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PEA MOSAIC AND ITS RELATION TO OTHER LEGUME MOSAIC VIRUSES[1]

By W. J. ZAUMEYER, *associate pathologist*, and B. L. WADE, *associate geneticist*, *Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

Pea disease surveys made in a number of Western States revealed the fact that mosaic was usually more prevalent along the borders of fields than elsewhere. The results of these surveys suggested the possibility that the legumes growing along irrigation ditches, roadways, and borders of fields might be the source of the viruses that cause the mosaic diseases of pea (*Pisum sativum* L.).

This paper embodies the results of a study of the common pea mosaic virus in comparison with viruses from red clover (*Trifolium pratense* L.), white clover (*T. repens* L.), white sweetclover (*Melilotus alba* Desr.), and alsike clover (*T. hybridum* L.). The paper presents (1) a description of the symptoms of the common pea mosaic, together with the symptoms of other legume mosaic diseases on their own hosts and on the pea; (2) preliminary data on the susceptibility and resistance of 42 varieties of peas to all these viruses; (3) methods for differentiating the viruses; and (4) data on the transmission of the common pea mosaic virus.

It seems probable that other viruses may be present in the various legumes which may sometimes produce reactions different from those recorded here. Likewise, viruses from other mosaic-infected legumes not included in this paper may produce symptoms on peas different from or similar to those here reported. Detailed work on virus properties may reveal that in some cases a given virus heretofore treated as a unit is composed of two or more separable components.

REVIEW OF LITERATURE

The first report of the occurrence of sweetclover and red clover mosaic was made by Elliott (*4*),[3] who showed that the virus was infectious to *Medicago arabica* (L.) Huds. and *Vicia faba* L. Dickson (*2*) reported that pea mosaic occurred in Quebec and that he had obtained successful inoculations from the garden pea to the sweet pea (*Lathyrus odoratus* L.), but negative results when cross inoculations to pea and bean were made. He also presented data on seed transmission of pea mosaic. Taubenhaus (*11*) reported the occurrence of sweet pea mosaic in Delaware and described a series of experiments which indicated that it was transmitted both by artificial inoculation and by aphids.

Doolittle and Jones (*3*) reported a mosaic disease of garden pea which they transmitted to red clover and sweet pea. They were

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[3] Reference is made by number (italic) to Literature Cited, p. 185.

unsuccessful in transmitting the virus of sweetclover and bean to pea and sweet pea.

Böning (*1*) described a mosaic disease of broadbean (*Vicia faba*), the virus of which was infectious to pea, crimson clover (*Trifolium incarnatum* L.), and red clover. He considered it as possibly identical with the pea mosaic described by Doolittle and Jones (*3*).

Merkel (*5*) believed that the same virus was responsible for the mosaic symptoms of the following hosts: *Phaseolus vulgaris*, *Pisum sativum*, *Lathyrus odoratus*, *Lupinus luteus* L., *Melilotus altissima* Thu., *Trifolium pratense*, *T. hybridum*, *T. repens*, *Anthyllis vulneraria* L., and *Vicia faba*.

Zaumeyer (*13*) and Zaumeyer and Wade (*14*, *15*) reported the transmission of a pea mosaic to bean (*Phaseolus vulgaris*). They also reported that a number of different legume mosaic viruses, including those of red clover, white clover, and white sweetclover, were transmissible to bean, pea, and sweet pea. Osborn (*7*, *8*) working on the transmission of two distinct viruses of pea, reported that one of them required an incubation period of about 18 hours in the aphids *Macrosiphum gei* Koch and *M. pisi* [*Illinoia pisi*] Kalt., while the other was transmissible within 30 minutes after it was picked up. Snyder (*10*) described a pea mosaic in California which produced serious pod deformation and which he believed was caused by a different virus from the common pea mosaic virus.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Pea mosaic has been found wherever peas are grown in the United States, and it is likely that the several legume viruses which are infectious to pea have equally as wide a distribution. Heretofore these diseases have not been differentiated, and since the symptoms produced by the various legume viruses on the pea are somewhat similar, it is not unlikely that some of the mosaic reported on pea was actually not the common pea mosaic. The virus diseases discussed in this paper apparently do not cause as much damage to pea varieties used for canning as they do to market-garden varieties. In sections where the latter are produced either for market or for seed the mosaic diseases are frequently epidemic, often causing considerable reduction in yield. Differences in varietal susceptibility possibly account in part for the greater damage to market-garden varieties.

Pea mosaic was first reported by Dickson (*2*) from Quebec, Canada, in 1922. Martin and Haensler [4] found it in New Jersey in 1924. Doolittle and Jones (*3*) found it in many commercial fields in Wisconsin in 1924, reporting that as many as 15 percent of the plants were infected. They also stated that Brotherton had reported it orally as occurring in considerable amount in Michigan in 1924. In 1928 Linford [5] reported it from nine States, from Utah to the Atlantic coast. It reached its maximum severity in New Jersey and Maryland. Snyder (*10*) described a pea mosaic in California in 1934 which caused considerable damage to the pods. The writers found it of considerable importance in certain sections of Colorado in 1932 and

[4] MARTIN, W. H., and HAENSLER, C. M. PEA DISEASES IN NEW JERSEY. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. 8: 43–53. 1924. [Mimeographed.]

[5] LINFORD, M. B. PEA DISEASES IN THE UNITED STATES IN 1928. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Reptr. Sup. 67: 1–14. 1929. [Mimeographed.]

1933, especially in peas grown at the higher altitudes. It was of less importance there in 1934 and 1935. Mosaic was also found in 1934 in Wyoming and southern Idaho and was reported to be common in sections of northern Idaho and Washington. It was observed more extensively in the market-garden varieties than in the canning and field varieties. Reports from the market-garden sections of the States of Washington, Oregon, and California indicate that it is of major importance there.

It appears that the disease, although not a serious factor in the curtailment of production except in certain localities, has become more widespread during the past few years and may be of some concern in the future. With the increased use of yellow and white sweetclovers as green-manure crops in certain of the western pea-seed-producing sections, it is possible that the mosaic of peas may increase because of the transmissibility of sweetclover mosaic to them.

Because of the similarity of symptoms of the common pea mosaic and the mosaics of related legumes on pea, little is known about the distribution of these various viruses. Experimental evidence has demonstrated that the common pea mosaic virus is carried in the seed in only a small amount, which indicates that seed transmission is usually not an important factor. Whether the viruses of other legumes inoculated to pea are carried in the seed in larger amounts is unknown.

Since aphids (*3*, *5*, *7*, *8*, *14*, *15*) have been shown to transmit the common pea mosaic virus, and since other closely related viruses are transmissible to pea, it is not unlikely that much of the infection found in a given locality is correlated with the aphid population and the number of infected legumes in the vicinity of the pea-producing areas.

MATERIALS

SOURCE OF SEED

The seed of the pea varieties used in the experimental work was either furnished by American seed firms or grown by the writers. Pea seed from mosaic-infected plants was obtained from three different seed firms.

SOURCE OF VIRUSES

Pea Mosaic

The common pea mosaic was collected from Dwarf Telephone pea plants grown in the southeastern part of Colorado, near Stonewall. Other material was secured from mosaic-infected Green Giant peas grown in Montana in 1933. The viruses from these two sources were identical. The experimental data presented here are based on the virus from Colorado. The virus extract from infected plants produces typical systemic mosaic symptoms (fig. 1, *A–D*, *H*, *I*, *K*, *L*), which are described later.

Red Clover Mosaic

The red clover virus used in these studies came from mosaic-infected plants collected near Rosslyn, Va. This virus is believed to be identical with the one described by Doolittle and Jones (*3*) as being infectious to pea and sweet pea. It produces only mottled symptoms when inoculated to pea.

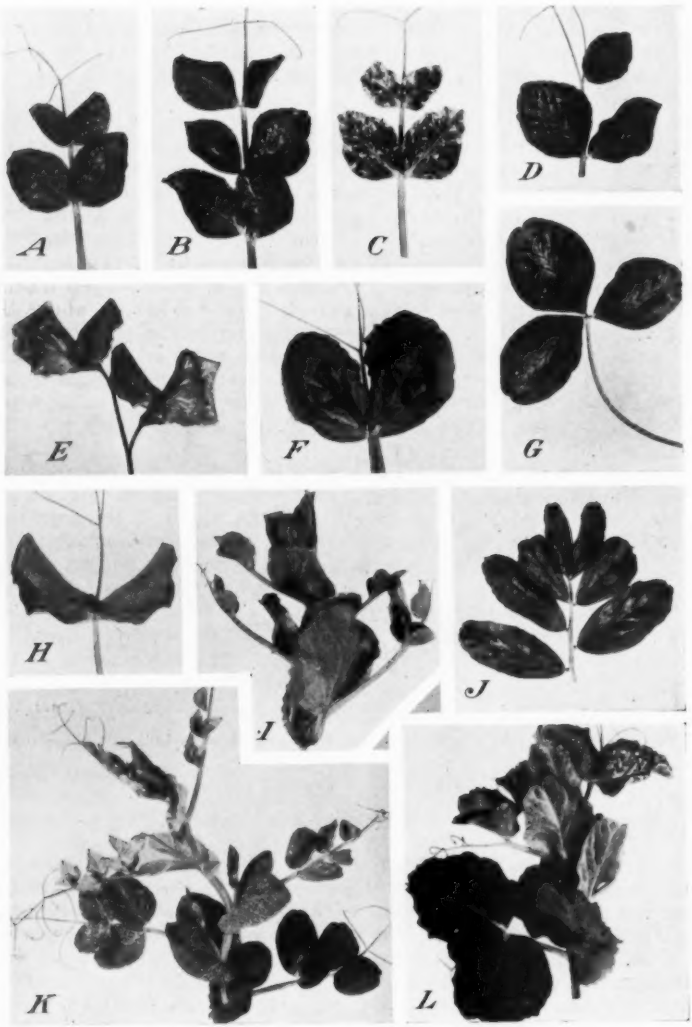

FIGURE 1.—Variations in leaf symptoms of pea mosaic on several pea varieties and of red clover mosaic on red clover, sweet pea, and *Vicia faba*: *A*, *B*, *C*, *D*, *K*, variations in symptoms on Green Giant peas from mild mottle to vein clearing and extreme chlorosis; *E*, *F*, mottling on flower and leaf of Baltimore Rose sweet pea; *G*, mottle on red clover; *H*, *I*, *L*, pea mosaic on Dwarf Telephone peas, showing inward curling, cupping, and delayed opening of leaves and stipules and heavy mottle and twisting of leaflets; *J*, leaves of *Vicia faba* showing mottling.

White Clover Mosaic

The virus of white clover mosaic was secured from material collected near Rosslyn, Va., and in the District of Columbia. The symptoms produced by both of these viruses were identical, both mottled and local necrotic symptoms appearing on many pea varieties (fig. 2, *D*, *E*, *F*).

White Sweetclover Mosaic

Collections of white sweetclover mosaic virus were made from white sweetclover plants grown near Rosslyn, Va., and in the District of Columbia. Like the white clover virus, it produces both mottled and necrotic symptoms (fig. 3, *A*, *D*, *E*, *F*, *G*, *J*, *K*). The mottled symptoms, however, were dissimilar to those produced by the white clover virus, while the necrotic lesions appeared identical (fig. 3, *K*).

Alsike Clover Mosaic

Specimens containing the virus of alsike clover mosaic were collected from alsike clover plants growing in northeastern Colorado. This virus produces only the mottled symptoms on peas, which are unlike those of the other viruses herein described (fig. 4). Other viruses from alsike clover, not discussed herein, show specific differences from this virus. For the sake of clarity, the virus reported herein will be designated as alsike clover virus 1.

METHODS

Most of the experimental work was carried on in the greenhouse, where the temperature varied from 18° to 25° C. The work on seed transmission was conducted both in the greenhouse at Rosslyn, Va., and in field plots in Colorado. In order that the different viruses might be readily available at all times they were transferred to *Vicia faba* plants, which served as a convenient host for culturing them.

The inoculum was prepared by grinding up mosaic-infected plants in a mortar. Plants were inoculated by briskly rubbing four to six leaflets with a cheesecloth pad saturated in the inoculum. They were not inoculated until they had grown to the eighth- to tenth-node stage. With this technique high percentages of infection were secured only with the white clover and white sweetclover mosaic viruses. The viruses of pea mosaic, red clover mosaic, and alsike clover mosaic were not readily transferred to peas.

Hoping to secure higher percentages of infection, especially for the studies of varietal resistance and susceptibility, the writers used the pea aphid (*Illinoia pisi* (Kalt.)) to transmit the red clover mosaic virus. The aphids were allowed to feed on *Vicia faba* infected with the red clover virus and later were transferred to healthy peas under cages. This method likewise failed to produce satisfactory infection.

Osborn (*8*) reports similar difficulties in securing transmission of pea mosaic by both the rubbing technique and the needle-prick method. Later studies, which are not reported in this paper, have shown that with the use of carborundum powder as an abrasive, higher percentages of infection may be obtained.

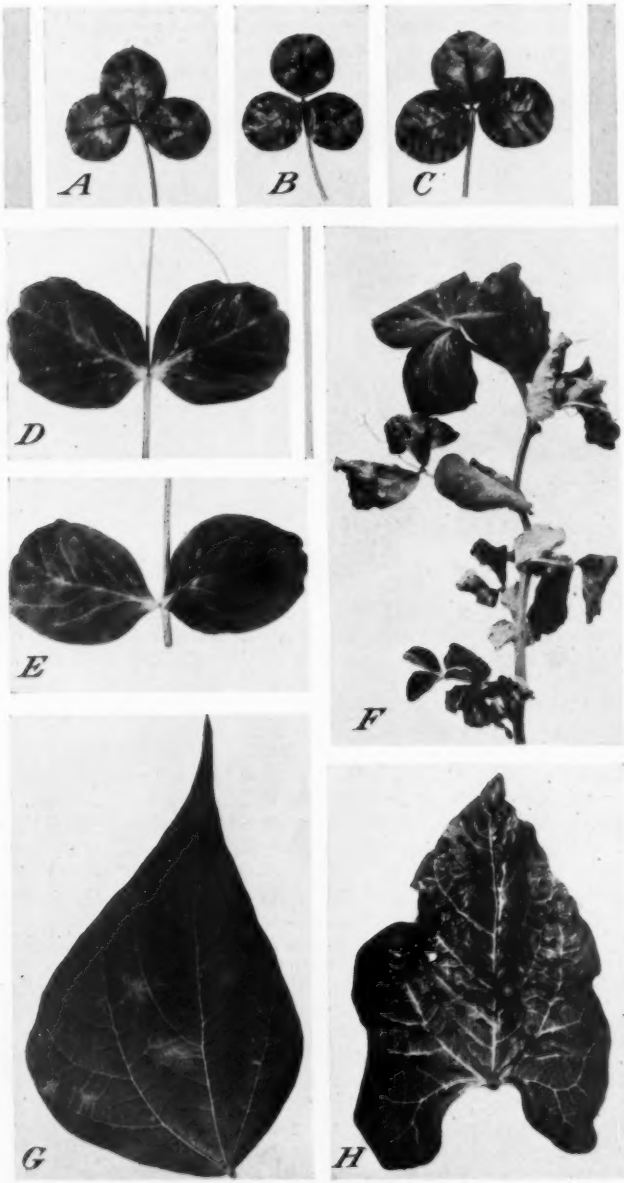

FIGURE 2.—Symptoms of white clover mosaic on white clover, peas, and beans: *A*, *B*, *C*, variations in mottle on white clover; *D*, *E*, mild mottle symptoms on Alderman pea; *F*, necrotic symptoms on Green Giant pea; *G*, *H*, systemic and local lesions on Stringless Green Refugee bean.

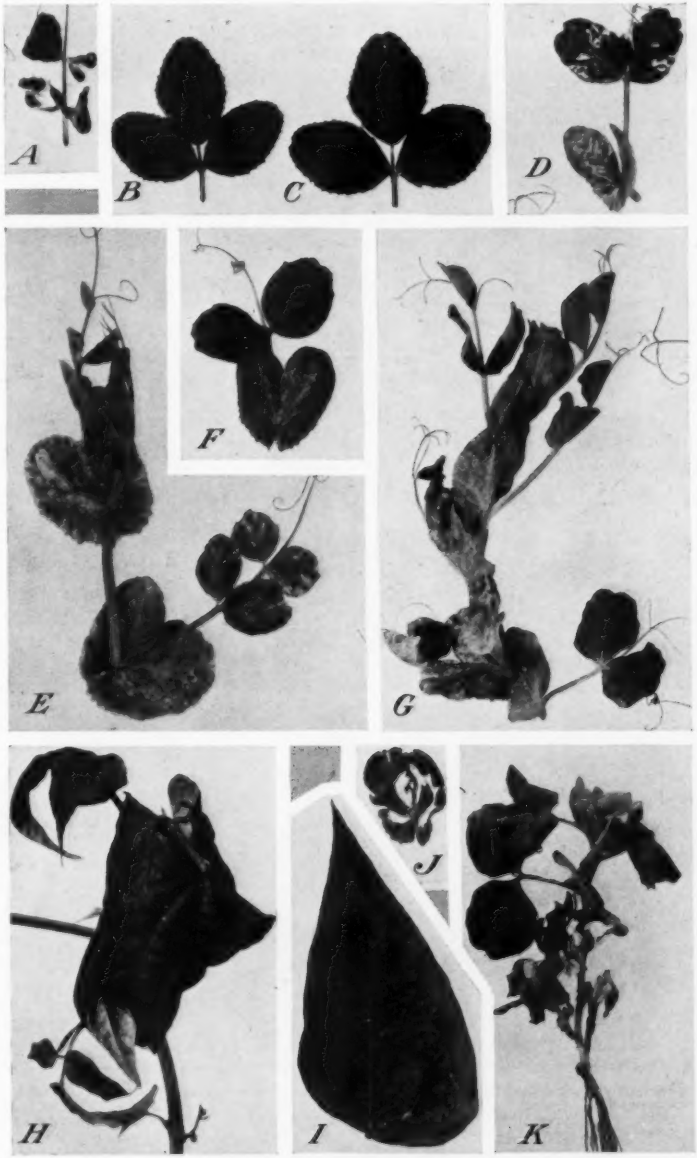

FIGURE 3.—Symptoms of white sweetclover mosaic on white sweetclover, peas, and beans: *A, D, F, J,* variations in mottle and chlorosis on Green Giant pea; *B, C,* variations in mottle on white sweetclover; *E,* mottle on Tall Telephone pea; *G,* mottle, delayed opening, and curling of leaflets on Bruce pea; *H, I,* downward curling of leaflets at the pulvini and mottle on Stringless Green Refugee bean; *K,* necrotic symptoms on Green Giant pea.

SYMPTOMS

The descriptions of the symptoms produced by the various viruses on the pea are based on the symptoms as they appear on the Dwarf Telephone and Green Giant varieties artificially inoculated in the

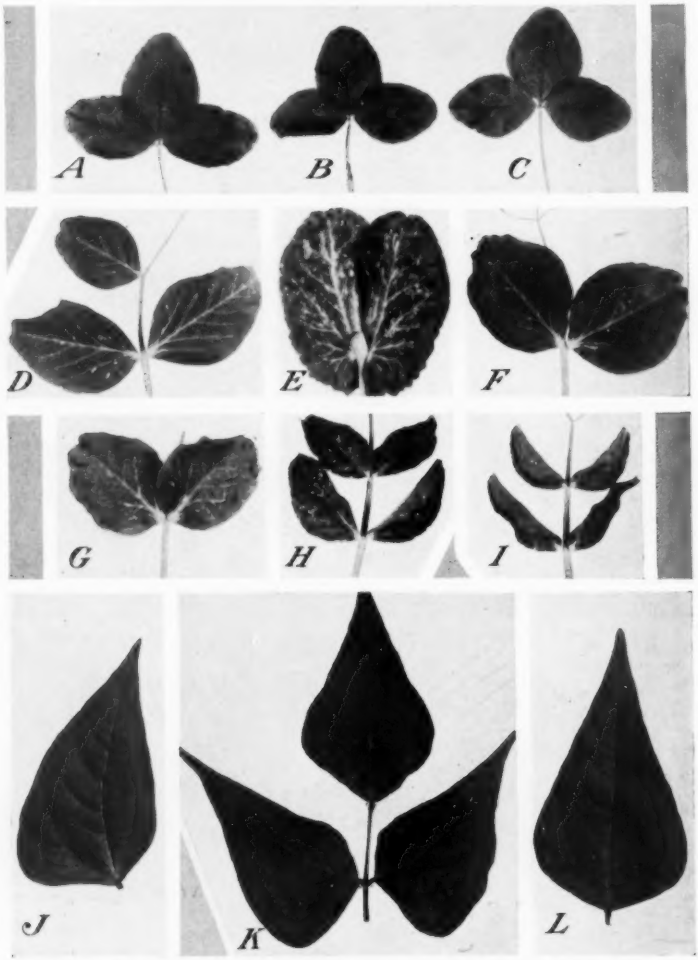

FIGURE 4.—Symptoms produced by the alsike clover mosaic virus on various hosts: *A, B, C,* symptoms on alsike clover; *D, E, F, G,* variations in mottle symptoms on leaves and stipules of Horsford pea; *H, I,* symptoms on leaflets of Tall Telephone pea; *J, L,* healthy leaves of Stringless Green Refugee bean; *K,* symptoms on Stringless Green Refugee bean.

greenhouse. In the case of alsike clover mosaic virus 1, the symptoms are described as they appear on the Tall Telephone variety. Although the symptoms produced by several of these viruses differ in certain

respects from one another, still there are certain overlapping similarities which make it difficult to differentiate clearly all of the viruses by symptomatology alone.

COMMON PEA MOSAIC

The first noticeable symptom produced by the virus of the common pea mosaic appears on the leaves and stipules (fig. 1, *A–D*) above the point of infection as a very slight chlorosis, the leaves being lighter green than normal. Very striking characteristics of young infected plants are the failure of the leaves to unfold as quickly as the leaves of an uninfected plant and the slightly wavy leaf edges (fig. 1, *H*, *I*). Later a mottling appears, due to the presence of small indistinct yellow spots located between the veins. The yellow areas increase in size, and dark-green irregularly shaped areas appear between the larger veins, producing an intense mottle. A pronounced vein clearing often appears (fig. 1, *K*), which increases in intensity from the lower to the upper branches. The tissue between the veins may vary from a moderately dark to a very light green to yellow. Infrequently, instead of a vein clearing, the green tissue persists in the region immediately adjacent to the veins and veinlets (fig. 1, *D*), while the area between the veins may vary from a light green to a yellow.

In the case of plants from infected seed, the symptoms are very pronounced. In certain cases an entire leaflet may be devoid of chlorophyll, with only a small amount of green pigment present at the base of the petiole (fig. 1, *K*). In still other instances all the leaves and stipules of a branch may be yellow, such leaves always being smaller than normal (fig. 1, *K*). Where a leaflet is only partially etiolated it may be decidedly malformed, wrinkled, puckered, or blistered, with the edges curled upward (fig. 1, *I*, *L*). The uppermost leaves of a severely infected plant do not open as readily as those of a normal plant, and often they remain folded tightly together. The symptoms are always manifested more clearly in the uppermost part of the plant (fig. 1, *L*).

When the infection occurs early in the life of the plant a stunting may result, but when it occurs later little reduction in size of the plant is noted. The pods of an infected plant may be somewhat malformed and distorted and often reduced in size.

RED CLOVER MOSAIC

The symptoms of the red clover mosaic virus first appear on the young center leaves of red clover as a mild mottle (fig. 1, *G*). Later the leaves become slightly streaked with yellow, which may sometimes extend over considerable portions of the leaves, with small islands or larger sectors of green tissue remaining. The islands of green, which are usually linear in shape, are frequently located adjacent to the veins. These green areas may follow a single vein or they may extend over three or four veins. Occasionally, in the case of severe infection, the leaves may show an arching with the midvein depressed. Stunting of infected plants is not common, and under conditions of high temperature the symptoms are readily masked.

RED CLOVER MOSAIC ON PEA

The symptoms produced by the virus of red clover mosaic when inoculated to pea are indistinguishable from those produced by the common pea mosaic virus.

WHITE CLOVER MOSAIC

The initial symptom of white clover mosaic is a very mild stipple-like mottle of the leaflet due to the presence of small light-green areas between the veins (fig. 2, *A*, *B*, *C*). Later the leaflet shows a more distinct mottle of light- and dark-green areas. The light-green areas enlarge and take on a light yellowish color, which may produce a considerable streaking of the leaves. The streaks sometimes follow the veins and may extend from the midrib to the margin of the leaflet. In other cases a considerable portion of the leaflet may be chlorotic, the remaining green areas usually being found adjacent to the midrib and extending outward toward the laterals. Islands of dark-green tissue frequently occur in other portions of the leaflet, either along the periphery or particularly at the base. Infected leaves are usually not distorted, but in some cases they are smaller than the normal leaves. Environmental conditions such as low or extremely high temperatures readily mask the symptoms.

WHITE CLOVER MOSAIC ON PEA

The white clover mosaic virus when inoculated to pea produces necrotic lesions and mild mottling. The necrotic lesions appear in about 7 to 10 days after inoculation. The first symptoms are a drooping of the inoculated leaves (fig. 2, *F*), a slight yellowing of the entire plant, and the eventual death of the inoculated leaves. The midvein on the upper side of the stipule nearest the point of inoculation takes on a purplish hue, and most of the veins on the lower side of the leaflets become discolored. The stipules recurve and the leaflets stiffen and curve downward. The uppermost leaves are subnormal in size, do not unfold as readily as normal leaves, and take on a crinkled appearance. The stems shrivel and turn to an indistinct purple, while the lower leaves shrivel and die. The vascular bundles of infected stems become discolored and necrotic; fungi may be isolated from them occasionally, but in no case have they proved pathogenic. It is likely that infected plants become weakened by the virus and that saprophytic fungi enter the root system. Infected plants often die, but it is believed that death does not result directly from the virus but from the virus in conjunction with organisms found in the roots and stems.

Plants infected with the white clover virus are not stunted, and the symptoms may be readily overlooked. The mottled symptoms are first manifested by small light-green areas appearing between the veins (fig. 2, *D*, *E*). They are not readily observed except by transmitted light. Later these leaflets become slightly chlorotic and blotches of light green appear, which are not bounded by the veins but extend throughout the entire leaflet. The infected leaflets are normal in size and are not malformed. Sometimes chlorosis may occur only along the edges of the leaves, the normal green remaining along the midvein and part way along the branch veins. More frequently the symptoms are noticeable only toward the base of the plant, the upper leaves appearing normal.

WHITE SWEETCLOVER MOSAIC

The first symptoms of white sweetclover mosaic appear as small, somewhat indistinct light-yellow spots on the leaves. These spots enlarge, coalesce, and may finally cover almost the entire leaflet, producing a speckled effect (fig. 3, *B*, *C*). The infected leaflet may become quite chlorotic with only small areas of green remaining, usually adjacent to the midvein and to some of the laterals. In other cases there may be a clearing of the veins with darker-green areas between them. Frequently the portion of the leaflet immediately adjacent to the midrib and extending some distance from it may be cleared, leaving only portions of the leaf margin a darker green. Under greenhouse conditions, especially in cases of severe infection, the leaves may be dwarfed and ruffled. In the field the infected plant may be stunted early in the spring, but later in the season it may be almost normal in size and the symptoms may be masked, especially if temperatures are high.

WHITE SWEETCLOVER MOSAIC ON PEA

The white sweetclover mosaic virus is similar to the white clover mosaic virus in that it produces both the necrotic and systemic symptoms on pea. The necrotic lesions are essentially identical with those produced by the white clover virus and it is impossible to differentiate them. The mottled symptoms, however, are decidedly different, and it is in this respect that the two viruses can be separated. The virus makes its first appearance as small, indistinct light-green spots located between the veins. These later enlarge and become more distinct and the entire leaflet may be mottled (fig. 3, *E*, *F*, *G*). Finally a light green replaces the dark green in the tissue except the dark green adjacent to the veins (fig. 3, *E*), which may persist. The light-green areas finally become yellow, producing a marblelike effect. The lower leaves may appear entirely normal at a distance of four nodes from the point of inoculation. The uppermost leaves, on the other hand, are smaller, sometimes greatly reduced in size, decidedly puckered, blistered, and twisted, often with an upward curl of the leaf periphery (fig. 3, *G*). Some of the leaves may have large blotches of yellow interspersed with areas of green (fig. 3, *A*, *D*, *J*). The yellow tissue apparently fails to develop normally, and with the continued growth of the cells in the normal green tissue the infected leaflets become distorted and malformed. Occasionally one stipule may be almost normal, whereas the opposite one may be greatly distorted and remain decidedly reduced in size. In severe cases of infection, the plant may be dwarfed, and in such instances the pods fail to develop normally.

In certain respects the mosaic symptoms produced on pea by the white sweetclover virus are somewhat similar to those of pea mosaic. However, the production of large yellow blotches in the youngest leaves, accompanied by a decided malformation of these leaves, provides a means whereby the white sweetclover virus can be differentiated from the pea mosaic virus. In mild cases of infection the symptoms may be similar to those produced by the white clover virus.

ALSIKE CLOVER MOSAIC

The symptoms manifested by the alsike clover virus 1 (fig. 4, *A*, *B*, *C*) are essentially the same as those of white clover mosaic.

ALSIKE CLOVER MOSAIC VIRUS 1 ON PEA

Alsike clover mosaic virus 1 produces only the mottled symptoms on pea. In this respect it differs from the white clover virus, which it resembles, and from the white sweetclover virus. The symptoms are first noted as a mild stippling of the leaves (fig. 4, *D–G*), produced by small light-green areas interspersed among the normal green of the leaflet. Only a slight chlorosis is produced, and frequently the darker green tissue is more noticeable along the veins and veinlets (fig. 4, *F*, *G*). The infected leaves and stipules are normal in size and are not malformed. These characteristic features differentiate it from the pea and red clover viruses, which produce marked symptoms on Green Giant and Dwarf Telephone varieties, as described previously (fig. 1).

On other varieties, such as Tall Telephone, the alsike clover mosaic virus 1 produces very conspicuous symptoms, such as a decided chlorosis of the leaves and stipules with only small areas of light-green tissue remaining. These areas are usually found at the base of the leaflet and frequently adjacent to the vein and veinlets. In other instances these darker areas are interveinal and appear as blotches or small islands of green. Infected pea plants are not stunted as they are when infected with the white sweetclover virus. The infected leaflets do not unfold normally but are cupped upward (fig. 4, *H*, *I*), especially toward the tip of the leaflet. Such leaflets are smaller than normal, but are not otherwise malformed.

PEA VARIETAL RESISTANCE AND SUSCEPTIBILITY

The several viruses infectious to pea are not readily transmitted by the usual mechanical methods. Because of this the results here reported are not entirely conclusive. However, they do indicate that certain varieties are decidedly more resistant than others.

Forty-two varieties of peas were inoculated with the several viruses under greenhouse conditions. Although it is believed that the red clover mosaic virus is similar to if not identical with the pea mosaic, it was included in these studies. The plants used in this test were grown in greenhouse benches and inoculated with an undiluted extract from the various mosaics according to the method already described. In addition to inoculation by this method, the red clover virus was also transmitted to peas by means of the pea aphid. The viruses used in the mechanical inoculations were either extracted from mosaic tissues of the original host plant or from peas or *Vicia faba* infected with the various mosaic viruses. The results in all cases were comparable. All of the varieties tested were not inoculated at one time, but were run in several series. As may be seen from table 1, the varieties were divided into three general groups, according to use and season.

TABLE 1.—*Susceptibility and resistance of different varieties of pea to mosaic viruses of pea, red clover, white clover, white sweetclover, and alsike clover*

Group and variety	Results of inoculation [1] with virus indicated															
	Pea mosaic, mottled		Red clover mosaic, mottled				White clover mosaic				White sweetclover mosaic				Alsike clover mosaic 1, mottled	
			Mechanical transmission		Aphid transmission		Mottled		Necrotic		Mottled		Necrotic			
	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]	Plants inoculated	Plants infected [2]
Canning:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Early:																
Alaska	11	6*a*	20	1*c*	11	0	22	3*b*, 6*c*	25	18*a*, 5*b*, 2*c*	32	6*a*, 11*b*	10	6*a*, 4*c*	31	7*a*, 4*b*
Surprise	10	0	11	0	10	2*c*	29	8*c*	29	3*b*, 16*c*	29	0	29	15*a*, 3*b*, 6*c*	10	0
Wisconsin Early Sweet	10	0	10	0	12	0	10	0	6	5*b*, 1*d*	10	0	10	10*c*	10	0
Midseason:																
Bruce	9	1*a*	12	0	10	4*a*, 4*b*	10	10*c*	10	6*c*	10	3*a*	10	10*c*	11	3*b*
Green Admiral	10	7*b*	9	0	10	7*b*	24	23*c*	26	1*b*, 22*c*	12	5*b*, 4*c*	12	1*b*, 5*c*	9	3*b*
Green Giant	28	4*a*	15	0	12	3*a*	21	7*b*, 12*c*	46	3*a*, 31*b*, 5*c*, 7*d*	20	15*c*	43	13*b*, 13*c*, 17*d*	23	10*b*, 2*c*
Horsford	9	1*a*	10	0	10	8*a*	10	10*b*	10	5*c*	10	6*c*	10	0	9	8*c*
Perfection	10	0	9	1*c*	8	0	27	9*b*, 12*c*	27	1*a*, 1*b*, 23*c*	20	8*b*, 12*c*	20	2*a*, 7*b*, 7*c*	10	1*c*
Perfection (wilt resistant)	9	3*c*	10	0	11	0	25	21*c*	20	8*a*, 10*b*, 2*c*	20	7*b*, 12*c*	20	1*a*, 18*b*, 1*c*	8	8*c*
Prince of Wales	10	3*b*	8	0	12	3*c*	10	10*c*	10	10*b*	10	1*a*, 9*c*	10	10*b*	11	1*a*, 2*b*
Senator	10	3*b*	10	0	10	4*a*, 5*b*	10	8*c*	10	10*b*	10	5*c*	10	10*b*	10	0
Yellow Admiral	10	2*b*	9	0	10	3*a*, 3*b*	10	9*c*	10	10*b*	10	6*c*	10	10*c*	10	8*c*
Market garden:																
Early:																
Bliss Everbearing	14	7*a*	10	0	11	2*a*, 2*c*	16	14*c*	15	10*c*	17	3*a*	18	7*c*	8	1*b*
Extra Early	8	5*c*	12	4*c*	12	5*c*									11	1*c*
Gradus	10	2*a*	10	2*c*	10	10*a*	24	12*c*	24	14*a*, 10*b*	17	8*b*, 9*c*	16	7*a*, 9*c*	10	5*a*
Hundredfold	10	0	10	0	10	0	12	6*b*, 4*c*	12	2*b*, 10*c*	20	6*b*, 7*c*	32	9*a*, 1*b*, 5*c*	9	0
Laxtonian	10	3*c*	9	0	14	0	18	9*b*, 4*c*	18	8*a*, 10*b*	10	2*a*, 2*b*	8	8*a*	13	1*b*, 2*c*
Laxton Progress	12	1*a*	7	4*b*	8	6*a*	27	10*c*	34	22*b*, 5*c*, 7*d*	24	4*c*	31	10*b*, 11*c*, 7*d*	10	2*b*
Laxton Superb	9	6*c*	10	10*c*	10	0	16	8*c*	17	8*a*, 8*b*, 1*c*	24	3*b*, 17*c*	24	9*a*, 11*b*, 1*c*	10	4*c*
Little Marvel	8	0	11	0	12	0	20	9*c*	20	5*b*, 8*c*	24	2*b*, 17*c*	26	4*a*, 14*b*, 3*c*	10	4*c*
Nott Excelsior	10	0	10	0	10	0	10	6*c*	10	9*c*	19	1*c*	19	6*c*	17	0
Pioneer	8	4*b*	10	1*c*	9	6*b*										

[1] All plants were inoculated by mechanical transmission except those inoculated with red clover mosaic virus, in which case transmission was made both mechanically and by means of aphids.

[2] *a* represents severe symptoms; *b*, moderate symptoms; *c*, mild symptoms; and *d*, death of plant.

TABLE 1.—*Susceptibility and resistance of different varieties of pea to mosaic viruses of pea, red clover, white clover, white sweetclover, and alsike clover*—Continued

Group and variety	Results of inoculation with virus indicated															
	Pea mosaic, mottled		Red clover mosaic, mottled				White clover mosaic				White sweetclover mosaic				Alsike clover mosaic 1, mottled	
			Mechanical transmission		Aphid transmission		Mottled		Necrotic		Mottled		Necrotic			
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Market garden—Continued.	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Early—Continued.																
Premium Gem	10	0	10	0	9	0	10	10*b*	10	7*a*, 3*d*	9	8*c*	10	5*a*, 4*c*, 1*d*	10	0
Pride of the Market	10	6*a*	10	0	10	5*b*	13	8*c*	13	5*b*, 8*c*	10	6*a*, 4*c*	10	9*c*	9	2*a*, 3*b*, 2*c*
Thomas Laxton	10	0	12	3*c*	10	1*b*, 2*c*	33	2*c*	45	9*a*, 32*b*, 4*c*	19	3*a*, 1*c*	35	11*a*, 14*b*, 10*c*	21	8*b*
World Record	10	1*a*	10	4*c*	10	5*a*	34	26*c*	34	28*a*, 5*b*	21	1*a*, 12*c*	22	8*a*, 12*b*, 1*c*	10	3*a*
Midseason:																
Alderman	10	7*a*	11	3*c*	10	5*a*	32	22*c*	32	17*a*, 15*b*	18	11*c*	26	16*a*, 10*b*	11	5*b*
Champion of England	10	4*a*	11	6*b*	9	3*a*, 3*b*	10	4*c*	7	5*b*, 1*c*	6	2*b*, 1*c*	6	5*b*, 1*c*	10	8*c*
Dwarf Alderman	10	2*a*	10	2*c*	10	0	8	0	21	12*b*, 9*c*	10	10*c*	15	7*b*, 7*c*	10	8*b*
Dwarf Quite Content	18	5*a*	----	----	12	5*a*	10	0	10	5*a*, 5*d*	10	2*a*, 3*c*	9	2*a*, 3*c*, 3*d*	9	4*b*, 3*c*
Dwarf Telephone	26	13*a*, 6*b*	10	2*b*	10	1*a*, 2*b*	19	8*b*, 11*c*	36	32*b*, 4*c*	16	2*a*, 14*b*	33	14*b*, 17*c*	29	13*b*
Giant Stride	10	6*a*	10	2*c*	10	5*a*	10	4*c*	10	2*c*	13	1*c*	13	3*c*	10	3*c*
Lincoln	18	3*a*	10	3*c*	15	11*a*	15	4*c*	15	10*b*, 5*c*	15	5*b*, 5*c*	16	4*b*, 12*c*	18	2*b*, 5*c*
Onward	10	3*c*	10	0	12	0	10	10*b*	10	2*b*, 8*c*	9	9*c*	9	7*c*	9	0
Phenomenon	8	2*a*	----	----	11	2*b*	10	9*b*	25	3*a*, 4*b*, 6*c*	9	6*a*	27	4*a*, 17*b*, 4*c*	10	1*a*
Profusion	10	0	----	----	10	8*c*	10	10*c*	10	10*c*	8	1*a*, 6*c*	8	8*c*	16	1*b*, 1*c*
Stratagem	10	6*a*	7	0	10	8*a*	12	10*b*	30	12*b*, 13*c*, 5*d*	10	4*b*, 6*c*	25	10*b*, 13*c*	13	4*b*
Tall Telephone	10	1*a*, 6*b*	----	----	11	6*b*	20	20*c*	39	18*a*, 21*b*	10	10*c*	15	7*b*, 7*c*	10	8*b*
Field:																
Austrian Winter	11	1*c*	10	3*c*	16	3*c*	11	11*c*	11	11*c*	11	11*c*	11	4*c*	10	2*a*
Black Eye Marrowfat	12	3*b*	11	2*c*	10	4*b*	10	3*c*	10	10*c*	10	1*a*, 3*b*	10	7*b*, 3*c*	12	5*b*
Maple	10	4*a*	12	0	11	3*c*	10	10*c*	10	6*c*	10	2*a*, 3*c*	10	10*c*	----	----
White Eye Marrowfat	10	0	11	1*b*	10	0	10	6*c*	10	9*c*	10	1*a*, 3*b*, 2*c*	10	8*b*, 2*c*	10	9*c*

It is believed that varieties showing 50 percent or more of mosaic when inoculated artificially would be considered quite susceptible under field conditions, while those showing less would be considered moderately susceptible. In certain instances only a small percentage of mosaic-infected plants may be noted, but the symptoms may be very severe. In other instances a large percentage of the plants inoculated may show only mild symptoms.

PEA MOSAIC

Table 1 shows that of the 12 varieties inoculated in the canning group, 3 were not infected, 1 showed mild, 4 moderate, and 4 severe symptoms. Of those showing no infection, Surprise and Wisconsin Early Sweet are early varieties and Perfection is a midseason variety. Alaska, Bruce, Green Giant, and Horsford showed marked symptoms, but only in the Alaska were more than 50 percent of the plants infected.

In the 26 market-garden varieties, 6 were not infected, 1 showed moderate, 4 mild, and 13 severe symptoms, and 2 varieties showed both severe and moderate symptoms. Of the varieties that manifested severe symptoms, the following six showed 50 percent or more of infection: Bliss Everbearing, Pride of the Market, Alderman, Giant Stride, Dwarf Telephone, and Stratagem. In the field group the White Eye Marrowfat showed no infection while Maple exhibited severe symptoms. The Austrian winter and Black Eye Marrowfat showed mild and moderate symptoms, respectively.

RED CLOVER MOSAIC

In general the pea varietal reaction to the red clover mosaic virus was somewhat similar to the reaction to pea mosaic, which tends to substantiate the belief that these two viruses are similar. The results of the mechanical transmission are not comparable in every case with aphid transmission, since more infection resulted from the latter method than from the former. In the canning group, two varieties were not infected by either method, four showed mild symptoms, one moderate, and two severe. In three varieties both severe and moderate symptoms were noted. In the market-garden group six varieties were not infected by either method, four manifested mild symptoms, three only moderate symptoms, and two only severe symptoms, while two varieties showed both mild and moderate symptoms, three both moderate and severe, and six both mild and severe symptoms. In the varieties classified as field peas no resistance was noted. All varieties showed mild or moderate symptoms.

There appears to be some correlation between the susceptibility and resistance of the various varieties to the red clover and pea mosaic viruses. With the exception of Surprise, Perfection, Profusion, Thomas Laxton, and White Eye Marrowfat, all of the ten varieties showing no infection with the pea mosaic virus likewise were not infected with the red clover virus. These five varieties were only slightly susceptible to the red clover virus, and the symptoms produced were in general very mild (table 1). The wilt-resistant Perfection, Laxtonian, and Onward varieties, were mildly infected by the pea mosaic virus, but were not infected by the red clover virus. Since these minor variations in susceptibility and resistance are not

outstanding, they do not appear to indicate specific differences in the two viruses, but are probably due to a combination of factors, such as age of inoculated plants, differences in concentration of the virus used, or slight variations in inoculation methods. Practically all the varieties manifesting marked symptoms when infected with one virus also produced similar symptoms when infected with the other. The Alaska variety, however, showed only a small percentage of infection when inoculated with the red clover virus, but was quite susceptible to the pea mosaic virus.

WHITE CLOVER MOSAIC

Of 40 pea varieties inoculated with the white clover mosaic virus only 3, Wisconsin Early Sweet, Dwarf Quite Content, and Dwarf Alderman, showed no mottled symptoms. Very mild symptoms were produced in almost all cases, and the plants were not stunted. All of the other varieties except Alaska, Surprise, Little Marvel, Laxton Progress, Thomas Laxton, Giant Stride, Champion of England, Lincoln, and Black Eye Marrowfat were very susceptible. The only correlation between resistance to this virus and to the mosaic viruses of pea and red clover is found in the Wisconsin Early Sweet, which is resistant to the three viruses.

In addition to the mottled mosaic symptoms, the white clover virus also produced marked necrotic lesions on the stems and inoculated leaves. All of the varieties tested exhibited these symptoms, and in some cases the virus caused death of the plant. Giant Stride was the only variety that showed some tolerance. Although showing a high percentage of infected plants, all of the field varieties manifested only mild symptoms.

WHITE SWEETCLOVER MOSAIC

The white sweetclover mosaic virus, similar to the virus of white clover, produced both the mottled and necrotic symptoms when inoculated to peas. The mottled symptoms were noted on 38 varieties, while 2, Surprise and Wisconsin Early Sweet, both belonging to the canning group, were not infected. Three varieties, Laxton Progress, Nott Excelsior, and Giant Stride, showed a high degree of tolerance to the mottle symptom, 30 varieties were very susceptible, and 5 exhibited severe symptoms but showed less than 50-percent infection. Severe symptoms alone were noted in three varieties, while eight showed both severe and mild symptoms or severe and moderate symptoms. All other varieties manifested mild symptoms.

The Horsford was the only variety that did not develop necrotic lesions. All others except Bliss Everbearing, Nott Excelsior, Giant Stride, and Austrian Winter showed a high percentage of infection. Green Giant and Laxton Progress each showed an exceedingly high percentage of infection, ranging from mild symptoms to death of plants. One variety produced only severe symptoms, and in 14 varieties the symptoms varied from severe to less marked. In 11 varieties only mild symptoms were noted.

ALSIKE CLOVER MOSAIC 1

The varietal reaction of peas to the alsike clover mosaic virus 1 was somewhat similar to that of peas to the mosaic viruses of pea and

red clover. In the canning group three varieties, Surprise, Wisconsin Early Sweet, and Senator were not infected. In the market-garden group four varieties manifested no infection, while in the field group no resistance was shown by any variety. Of the seven noninfected varieties, four were not infected by the mosaic viruses of pea and red clover, one was not infected by the white clover mosaic virus which causes the mottled symptoms, and two were not infected by the virus of white sweetclover mosaic. Of the 33 susceptible varieties only 11 showed 50 percent or more infection. Severe symptoms alone were observed in four varieties, while in three both severe and milder symptoms were noted; of these seven varieties, five were in the class showing a low percentage of infection.

SUMMARY OF VARIETAL TESTS

The data presented in table 1 show that the viruses of white clover and white sweetclover are more infectious to pea than are the other viruses. The data also show that the highest percentage of noninfected varieties belong to the canning group. Of the 12 varieties in this group, the following number of varieties showed no infection: 3 when inoculated with pea mosaic virus, 2 with red clover virus, 1 with the white clover virus which produces mottled symptoms, 2 with the white sweetclover virus which produces mottled symptoms, and 3 with alsike clover virus 1. Of the 26 market-garden varieties, the following number of varieties were not infected: 6 by the pea virus, 6 by the red clover virus, 2 by the white clover virus which produces mottled symptoms, and 4 by the alsike clover virus 1. In the field group, comprising four varieties, one, the White Eye Marrowfat, was not infected by the pea mosaic virus. The viruses of the other mosaics were infectious to all the varieties of this group.

DIFFERENTIATION OF THE VIRUSES

Although, in general, specific differences in symptoms are manifested when certain pea varieties are inoculated with the various legume viruses, there are some overlapping features which make it difficult at times to differentiate them clearly. Varietal variations as well as environmental conditions may alter the symptoms and make symptomatological separation of the viruses extremely difficult.

In an earlier paper by the writers (*15*) extensive tests were reported on the host range of the mosaic viruses of white clover, white sweetclover, red clover, and of a pea mosaic virus designated as pea mosaic 2, which was distinct from the virus of the common pea mosaic. Host-range studies were not attempted with the viruses of the common pea mosaic and the alsike clover mosaic. It seemed likely that the virus of the common pea mosaic would cause reactions similar to those produced by the red clover mosaic virus, because of the identity of symptoms and reactions of these two viruses on pea varieties. It was also believed that the effects of the alsike clover mosaic virus 1 would be similar to those of pea mosaic virus 2, which it somewhat resembles.

These earlier studies revealed that the host range of the several viruses showed considerable variation. Pea mosaic virus 2 infected *Phaseolus angularis* Wight, *Cicer arietinum* L., *Lathyrus odoratus*, *Lens esculenta* Moench, *Melilotus alba* Desr., *Trifolium repens*, and

Vicia faba. The white clover mosaic virus was infectious to all of these hosts except *P. angularis*, and in addition it infected *P. lunatus* L., *P. mungo* L., *Medicago sativa* L., *Trifolium pratense*, and *Vicia americana* Muhl. The white sweetclover virus was infectious to all of the hosts susceptible to the white clover virus and, in addition, to *Cajanus indicus* Spreng.; but it was not infectious to *P. lunatus* and *Trifolium repens*.

The virus of red clover mosaic was infectious to all the hosts that were susceptible to the white clover and white sweetclover viruses, with the exception of *Phaseolus lunatus*, *P. mungo*, *Cajanus indicus*, *Medicago sativa*, and *Melilotus alba*.

From the results just mentioned it is apparent that the various viruses tested have different host ranges. In general, the reaction of a given host to a number of viruses was the same as that expressed by symptomatology, but different hosts showed a variety of symptoms.

The writers (*14*, *15*) also demonstrated that several of the legume viruses are infectious to bean, producing symptoms which make it possible to differentiate them from one another as well as from the common bean mosaic. Furthermore, differences in susceptibility and resistance among certain bean varieties were shown to be helpful in distinguishing the several viruses. In addition to studies on varietal resistance and susceptibility in the pea, preliminary separational reactions of certain bean varieties (table 2) and *Vicia faba* served to establish specific differences in the six viruses reported in this paper. Property studies of certain of the viruses discussed in this paper have been previously reported (*15*).

REACTION OF BEANS TO THE DIFFERENT VIRUSES

The mosaic viruses of pea and red clover can be distinguished from those of white clover and white sweetclover and from alsike clover mosaic virus 1 on the basis of their ability to infect bean (*Phaseolus vulgaris*). The viruses of pea and red clover are not infectious to bean, while the others produce definite symptoms on the Stringless Green Refugee variety.

The white clover mosaic virus is the only one that produces local lesions on bean (fig. 2, *H*) and thus it can be separated from the viruses of white sweetclover and alsike clover mosaic 1 (*15*). Furthermore, it produces systemic symptoms that differ from those produced by the other viruses. The symptoms on bean are very mild and are characterized by blotches of light green or yellow on the trifoliate leaves (fig. 2, *G*). Infected leaves may be slightly ruffled. However, there is no retardation in the growth of the leaf, and it is not malformed as it is when infected with the other viruses (fig. 2, *G*). Infected plants are never stunted.

The white sweetclover mosaic virus is readily distinguished from the others by the very marked symptoms that it produces on beans. These are first manifested by a drooping of the leaf and leaflets at the pulvini (fig. 3, *H*), followed by numerous small yellow halolike chlorotic spots (fig. 3, *I*). These spots enlarge and coalesce, producing a decidedly chlorotic condition of the leaf. The young trifoliate leaves are smaller than normal leaves and are decidedly rugose; they droop and curve inward, the tip in some cases almost touching the lower surface of the leaf (fig. 3, *H*). Infected plants are frequently stunted, and in severe cases they may die.

Alsike clover virus 1 produces only systemic lesions on beans, and thus it can be distinguished from the white clover mosaic, which it resembles. Furthermore, the systemic symptoms are more severe than those produced by the white clover virus and much less pronounced than those produced by the white sweetclover virus. They are first manifested by small light-green to yellow chlorotic spots, which enlarge and which may produce considerable chlorosis, especially on the older leaves. On the younger trifoliate leaves these spots frequently show normal green centers resembling a symptom of ring spot. Often light-green areas are found adjacent to the veins and veinlets with dark-green areas between, but occasionally the dark-green tissue may persist along the veins (fig. 4, *L*). Frequently large blotches of light green to yellow appear over the entire leaflet, resembling the symptoms produced by the white clover mosaic virus. Later, however, they are more marked and severe than those produced by the white clover virus and are easily differentiated. Infected leaves are somewhat smaller than normal and are slightly malformed, quite chlorotic, and somewhat rugose. Infected plants are only slightly stunted, the leaves and leaflets do not droop at the pulvini, nor do the young trifoliate leaves curve inward as they do in plants infected with the white sweetclover virus. From the last-named characteristic and from lack of severity of the symptoms, the alsike clover virus 1 can be identified as different from the white sweetclover virus. The fact that it does not produce local lesions and that it does cause characteristic light-green to yellow spots and severer and more pronounced symptoms serves as a means of differentiating it from the white clover virus.

In addition to the Stringless Green Refugee variety, three varieties, the Corbett Refugee, Robust, and Great Northern Idaho No. 1, which are resistant to the common bean mosaic virus, were used as a means of identifying the several viruses on the basis of varietal susceptibility and resistance. The results of these inoculations are shown in table 2.

Table 2 shows that the viruses of the common pea mosaic and the red clover mosaic are not infectious to bean. As mentioned earlier these two viruses can be separated from the others by this characteristic. The white clover mosaic virus, in addition to being the only one that produces local lesions on beans, can be separated from the other viruses by the fact that it is infectious to Stringless Green Refugee, Corbett Refugee, and Robust varieties, but not to Great Northern Idaho No. 1. The difference noted between alsike clover virus 1 and the white sweetclover virus is that the former is not as infectious as the latter, nor does it produce, except on the Stringless Green Refugee, as severe symptoms as does the white sweetclover virus.

TABLE 2.—*Resistance and susceptibility of 4 bean varieties to the mosaic viruses of several different legumes*

Variety	Reaction to indicated virus									
	Common pea mosaic		Red clover mosaic		White clover mosaic		White sweetclover mosaic		Alsike clover mosaic	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]	Plants inoculated	Plants infected [1]
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Stringless Green Refugee	12	0	10	0	12	11*b*	10	10*a*	16	9*a*, 3*b*, 4*c*
Corbett Refugee	12	0	11	0	15	2*b*, 7*c*	10	4*a*, 4*b*,	10	5*b*, 3*c*
Robust	10	0	10	0	10	3*c*	10	5*b*, 3*c*	18	2*c*
Great Northern Idaho No. 1	10	0	12	0	10	0	10	5*b*	24	1*c*

[1] *a* represents severe symptoms; *b*, moderate symptoms; and *c*, mild symptoms.

REACTIONS OF VICIA FABA TO THE DIFFERENT VIRUSES

Vicia faba is susceptible to all of the viruses discussed here. Although the symptoms vary less than they do on beans, a few of the viruses can be differentiated by differences in symptoms and also by severity of infection.

Of all the viruses those of the common pea mosaic and the red clover mosaic produce the severest symptoms on *Vicia faba*, and they cannot be differentiated from each other. The first symptom is a slight chlorosis of the affected plant. Islands of green tissue next appear adjacent to the veins and veinlets and frequently between them (fig. 1, *J*). Infected leaves may show considerable blistering and puckering in these regions and are often decidedly malformed. They are usually smaller than normal, and their edges are wavy and curl upward, especially toward the top of the plant. Severely infected plants are usually stunted and decidedly chlorotic.

The viruses of the mosaics of white clover, white sweetclover, and alsike clover 1 produce symptoms similar to one another when inoculated to *Vicia faba*. Infected plants are only mildly chlorotic and the leaves are slightly distorted or malformed. Islands of green tissue are found adjacent to the veins, but they are not so large, numerous, or extensive as they are on plants infected with the viruses of pea and red clover mosaic. Infected plants are only slightly stunted.

TRANSMISSION

SEED

Whether the virus of pea mosaic is transmitted through seed is still a moot question. Dickson (*2*) reported considerable seed transmission in certain varieties grown in the greenhouse. Out of a total of 130 seeds of 6 varieties, 55 produced diseased plants. Doolittle and Jones (*3*) reported no seed transmission in over 2,081 plants, which they grew from seed collected from mosaic-infected pea plants. Merkel (*5*) reported 0.8 percent of seed transmission. He did not state the number of seeds tested. Van der Meulen (*6*) was unable to show that the virus was carried through the seed. Böning (*1*) obtained only 0.2 percent of mosaic in a total of 9,180 plants of *Vicia*

faba grown from seeds from mosaic-infected plants. Although it is assumed that the virus he worked with is identical with the mosaic reported by Doolittle and Jones (*3*), it cannot be said that seed transmission through *V. faba* is similar to that through the pea. It appears that Dickson (*2*) may have been dealing with a different virus from those of other workers, because of the large amount of seed transmission he reports.

In order to secure additional evidence, seed from mosaic-infected pea plants, obtained from Colorado, Wyoming, Idaho, Montana, and Washington, was tested. Seven varieties were represented. Most of the seed was planted in the greenhouse, which was fumigated at regular intervals to prevent any secondary spread by insects. The rest of the seed was planted in the field. The infected seed collected in Montana was divided into two lots, (1) seed produced below the point of visible symptoms and (2) seed produced above this point.

Since it is possible to confuse primarily infected plants with those that may have become infected secondarily in the field, the notes were taken as soon as the symptoms appeared. Primarily infected seedlings manifest the symptoms early by a mottling and a decided stunting. When the first data were taken no mosaic had appeared in any of the lots except those that originated from infected plants. On the basis of this evidence it seems that the infected plants observed were produced from infected seed and were not infected from a secondary source. Through cross-inoculation studies it was determined that the virus in question was that of the common pea mosaic. Table 3 shows the results of this study.

TABLE 3.—*Results from test on seed transmission of pea mosaic*

Variety	Seed from mosaic-infected plants grown in—	Where seed was planted	Plants produced	Plants infected with mosaic
			Number	*Number*
Alderman	Washington	Greenhouse	100	0
Green Giant	Montana	do	[1] 750	2
Do	do	Field	[1] 814	6
Do	do	Greenhouse	[2] 200	0
Do	do	Field	[2] 339	0
Dwarf Telephone	Colorado	do	118	3
Do	do	do	67	0
Potlatch (Improved Stratagem)	Idaho	Greenhouse	314	0
Rogers D	do	do	120	0
Dwarf Alderman	do	do	22	0
Prince of Wales	do	do	213	0
Total			3,057	11

[1] From seeds collected above point of noticeable infection.
[2] From seeds collected below point of noticeable infection.

In a total of 3,057 seedlings only 11 showed mosaic infection. Out of a total of 1,564 plants from seeds produced above the point of noticeable infection, 8 plants showed the typical mosaic symptoms. Of 539 plants grown from seeds produced below the point of visible infection, no mosaic was observed. From this limited evidence it appears that only seeds produced above the infection point may carry the virus. Since the virus was transmitted through the seed in only a comparatively small percentage of cases, these results are not conclusive, but the evidence indicates that there is at least a

great reduction in primarily infected seeds if the seed is collected below the point of infection.

No data are available on seed transmission through peas of the other legume mosaic viruses to which peas are susceptible. In one trial of 140 plants from seeds collected from mosaic-infected plants in Maryland in 1932 and grown in the greenhouse, 25 plants showed mosaic symptoms. Inoculation to bean indicated that this virus was similar to pea mosaic virus 2, described in an earlier paper (*15*). Although this test is not conclusive, because of the small number of seeds tested, it does appear that some of these viruses may be carried through pea seed, possibly in higher percentages than the virus of the common pea mosaic. Because of the similarity of symptoms of these various viruses on pea, it would be difficult to determine a particular virus except through cross-inoculation.

INSECTS

The transmission of pea mosaic by the pea aphid (*Illinoia pisi*) was reported by Dickson (*2*), Doolittle and Jones (*3*), and Böning (*1*). Osborn (*7, 8*) transmitted a pea mosaic virus by means of the pea aphid (*Macrosiphum* [*Illinoia*] *pisi*) and the potato aphid (*M. gei*). He was not able to prove the bean aphid (*Aphis rumicis* L.) to be a vector. The writers were likewise able to transmit the pea and red clover mosaic viruses with the pea aphid, thus confirming the results of the previous workers. Transmission of the virus of white sweet-clover was secured with the pea and bean aphids, while tests with the green peach aphid (*Myzus periscae* Sulz.) gave negative results. In a few tests the pea aphid did not transmit the white clover mosaic virus to peas.

DISCUSSION

With the discovery of the transmissibility of various legume viruses to pea, the pea mosaic problem naturally becomes more complex. Although it had been shown prior to these investigations that a virus from red clover mosaic was transmissible to pea (*3*) and to several other legumes (*1, 2, 4*), and that a virus of pea mosaic is infectious to red clover (*3*), it had not been shown that several other legume viruses when inoculated to pea would produce infection. In fact, earlier tests had shown that a virus from *Melilotus alba* was not infectious to pea (*3*).

It is believed that the investigations presented here are significant insofar as they show that the pea can be infected with several distinct viruses producing symptoms which may be difficult to differentiate under certain environmental conditions. The results further show that the pea virus complex, unlike that of many other crops, may consist of distinct viruses of related hosts and not necessarily strains of the pea mosaic virus. If the several viruses described in this paper had been isolated from peas, one would have been led to believe that several strains or types of pea mosaic exist. It is not unlikely that later studies may show this to be the case. Doolittle and Jones (*3*) believe that aphids migrating from red clover to peas act as carriers of the disease, thus assuming that the red clover mosaic virus they worked with may have been identical with the common pea mosaic virus. In the inoculation studies of these investigations the red clover virus collected from numerous sources gave reactions identical

with the common pea mosaic virus except in the case of one collection, which will be discussed later. The symptoms produced by the other virus collections when inoculated to pea were identical with the symptoms of the common pea mosaic. Furthermore, both viruses produced identical symptoms on *Vicia faba* and neither was infectious to bean. More extended studies on host relationship and virus properties are necessary before it can be definitely said that these two viruses are identical. From preliminary evidence, however, it may be assumed that they are at least very closely related.

The question arises as to whether the virus from red clover here reported is a true mosaic virus of pea or of red clover. Since legume mosaics undoubtedly originated a long time ago, such a question is not susceptible to direct proof. However, since red clover is a winter-hardy perennial and the pea a semihardy annual, it would seem that a virus originating in red clover would have a much better opportunity of surviving than one occurring in peas. Moreover, red clover plants are only slightly injured by the virus while peas may be seriously injured, indicating perhaps that evolutionary tolerance has been developing for a longer time in the red clover than in the pea.

The other viruses considered in this paper have not been found as commonly on peas under natural conditions, indicating that peas are probably not a natural or original host. On the other hand, most of the numerous collections of viruses made from a given clover host were found to be a common virus of that host, showing a tendency for a virus to be fairly specific to the host in question.

It should not be understood that all the viruses collected from red clover are identical, even though the symptoms may indicate this. In one case it was found that a virus from this host produced symptoms on beans different from any reported here. Since other mosaic viruses such as the white clover, white sweetclover, alsike clover, and alfalfa are transmissible to *Trifolium pratense* (*15*), it is possible that certain of these viruses may be present in this host and that cross-inoculation studies with such material would reveal results different from those expected. However, from numerous tests made with material from a number of different sources it is believed that the typical red clover mosaic virus is not infectious to bean.

Even though white sweetclover is susceptible to the viruses of white clover, alsike clover, and alfalfa, only one type of symptoms has been produced on peas by the white sweetclover virus from many collections. On certain varieties of peas the symptoms manifest themselves very mildly and resemble those produced by the white clover mosaic virus. In the course of these experiments material from numerous sources was used. The mosaic virus of yellow sweetclover (*Melilotus officinalis* (L.) Lam.) was not used in these studies, but since its reaction on bean as reported earlier (*15*) was identical with that of white sweetclover, it is believed that its reaction on peas would also be similar.

Since the several viruses are intertransmissible between the various legumes, it is difficult to classify them according to the host from which they are collected. Likewise it is not always possible to separate all of them by means of the symptoms they produce on peas. Since these viruses when inoculated to certain varieties of beans and *Vicia faba* show differences, both as to symptoms and as to susceptibility and resistance, an additional means is available for differentiat-

ing them. Further studies on host range and virus properties, now in progress, may show still other distinguishing features.

As mentioned earlier, Böning's (*1*) virus disease of *Vicia faba*, which he transmitted to red clover, crimson clover, and pea, was believed by him to be probably identical with the red clover and pea mosaic reported by Doolittle and Jones (*3*). Limited observations by the writers with a virus isolated from mosaic-infected *V. faba* seedlings grown from infected seed confirmed these results. It is also believed that these two viruses are probably identical with the mosaic viruses of pea and red clover reported here.

The streak disease of peas reported by Linford,[6] characterized by streaked and spotted brown necrosis of pods, stems, and leaves, is unlike any of the viruses discussed in this paper. Pierce's (*9*) bean virus 2 is very likely identical with the white sweetclover virus referred to here. Pierce's (*9*) alfalfa virus 2, which was reported as being infectious to pea and *Vicia faba* and the alfalfa mosaic described by Weimer (*12*) are dissimilar to the viruses dealt with here.

The pea mosaic which Osborn (*7*) described as producing leaf enations on both peas and crimson clover is different from any of the viruses here described. Osborn's (*8*) pea mosaic 2 appears to be similar to the viruses of the common pea mosaic and red clover mosaic discussed in this paper.

SUMMARY

Field surveys showed that pea mosaic is usually more severe along borders of fields than elsewhere, suggesting the possibility of the transmission of certain legume viruses to pea.

Cross-inoculation studies showed that the mosaic viruses of red clover, white clover, white sweetclover, and alsike clover are infectious to pea.

It is probable that other mosaic viruses may be found in the several legumes that may react differently from those described here, notwithstanding the similarity of symptoms.

Pea mosaic has been found wherever peas are grown, but the disease seems to be more serious in sections where the market-garden varieties are grown either for shipping as green peas or for seed.

Under certain environmental conditions and on certain varieties, the viruses of the several legumes may produce symptoms similar to those of the common pea mosaic. By the use of Dwarf Telephone, Tall Telephone, and Green Giant pea varieties, the mosaic viruses of white clover, white sweetclover, and alsike clover can be differentiated from the viruses of pea and red clover mosaics. The symptoms produced by the mosaic viruses of pea and red clover are similar, and it is believed that the two viruses are identical. In addition to the mottled symptoms produced by all of the legume viruses when inoculated to pea, the mosaic viruses of white clover and white sweetclover produced necrotic lesions on the inoculated leaves and stems.

Susceptibility and resistance to the several viruses were studied with 42 varieties of peas. Ten varieties were not infected by the pea mosaic virus and 8 were not infected by the virus of red clover mosaic. Three varieties out of 40 did not exhibit the mottled symptoms of the white clover virus, but all were susceptible to the necrotic lesions, only

[6] LINFORD, M. B. See footnote 5.

1 variety showing a high degree of tolerance. The virus of white sweetclover mosaic produced the mottled symptoms on 38 varieties and necrotic lesions on 39. Seven varieties were not infected by alsike clover virus 1. The highest percentage of varieties not infected by the various viruses was found in the canning group.

In addition to the production of symptomatological differences, the several viruses can be separated on the basis of the reaction of Stringless Green Refugee, Corbett Refugee, Robust, and Resistant Great Northern Idaho No. 1 bean, and of *Vicia faba*.

Out of a total of 3,057 seeds collected from mosaic-infected pea plants, 11 produced diseased seedlings. In 1,564 plants grown from seeds collected above the point of noticeable infection, 8 were diseased, while in 539 seedlings grown from seeds collected below the point of noticeable infection no mosaic was observed.

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PARIS GREEN AND ITS HOMOLOGUES AS INSECTICIDES AGAINST THE JAPANESE BEETLE[1]

By WALTER E. FLEMING, *entomologist*, and FRANCIS E. BAKER, *assistant entomologist, Division of Fruit Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

During the summer of 1934 an investigation was made to determine the value of paris green and certain of its homologues as stomach-poison insecticides against the adult Japanese beetle (*Popillia japonica* Newman).

The materials investigated are listed in table 1.[2] With the exception of paris green and copper crotonoarsenite, the materials were ground in a paint mill with bone glue, ethylene glycol, and ethyl alcohol to form a paste that would disperse readily in water. The paris green was passed five times through a colloid mill to obtain a fine dispersion in water. Copper crotonoarsenite did not require a wetting agent and, because of the small quantity available for experimentation, was ground with water in a mortar as required. The arsenious oxide and cupric oxide in these materials were determined by the regular procedure [3] and are given in table 1.

TABLE 1.—*Materials investigated and their arsenious oxide and cupric oxide content*

Material	Total As_2O_3 in dry material	CuO in dry material	Water-soluble As_2O_3 (dry basis)
	Percent	*Percent*	*Percent*
Fish-oil green	23.30	17.34	11.48
Soybean-oil green	23.68	19.02	1.15
Tung-oil green	29.84	19.89	.12
Rapeseed-oil green	30.28	19.76	7.53
Cottonseed-oil green	30.52	20.51	4.93
Copper stearoarsenite	31.38	20.72	4.29
Copper oleoarsenite	35.45	19.89	2.61
Paris green	35.52	29.90	1.94
Copper palmitoarsenite	38.59	22.10	4.88
Copper lauroarsenite	43.65	24.23	1.94
Copper crotonoarsenite	52.55	31.96	5.33

EXPERIMENTAL PROCEDURE

One liter of each spray was prepared to contain a definite quantity of the dry weight of paris green or one of its homologues, and placed in a glass tank of 1.5 liters capacity, which was equipped with a small mechanical agitator to keep the solids in suspension. The sprays contained 1.25, 2.5, 5, 10, 20, and 40 grams of dry material per liter, which were approximately equivalent to 1, 2, 4, 8, 16, and 32 pounds

[1] Received for publication Apr. 9, 1936; issued September 1936.

[2] The homologues of paris green were prepared and analyzed by F. E. Dearborn, of the Division of Insecticide Investigations. The preparation of these materials is described in the following publication: DEARBORN, F. E. HOMOLOGS OF PARIS GREEN. Jour. Econ. Ent. 28: 710–713. 1935. The paris green was purchased on the open market, and was analyzed by L. Koblitsky, of the Division of Fruit Insect Investigations.

[3] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. . . . Ed. 3, 593 pp., illus. 1930. Washington, D. C. See pp. 40–48.

to 100 gallons. The spray flowed by gravity to a commercial spray gun and was applied to foliage under a 20-pound pressure. Smartweed plants (*Polygonum pennsylvanicum*) in pots were placed on revolving disks, mounted on a revolving table, and passed slowly through a fan-shaped spray, so that each material was applied uniformly to the foliage.

After being sprayed the plants were allowed to dry and were then placed in special glass cages under controlled temperature, relative humidity, and light. The details of the environmental conditions and the procedure of testing stomach-poison insecticides on the Japanese beetle have been described by the writers.[4] In testing the insecticidal value of a definite concentration of a material, 1,000 freshly collected beetles that had been starved for 6 hours were distributed in five cages containing treated plants, 200 beetles in each cage; at the same time an equal number were confined with

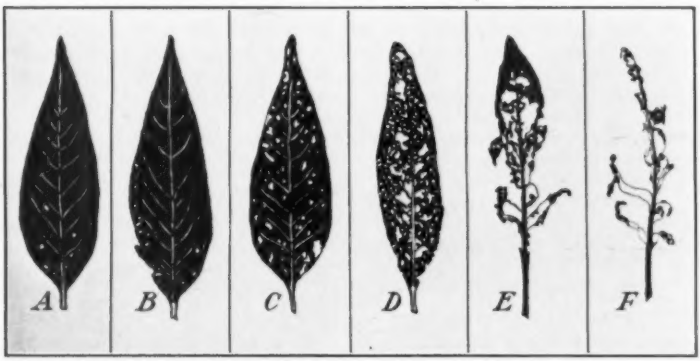

FIGURE 1.—The different degrees of feeding on foliage of smartweed by the Japanese beetle: *A*, Very slight; *B*, slight; *C*, moderate; *D*, extensive; *E*, very extensive; *F*, complete defoliation.

foliage sprayed with 8 pounds of acid lead arsenate and 2 pounds of light-pressed fish oil to 100 gallons of water to serve as a standard of insecticidal action, and an equal number were confined without food to determine the mortality caused by starvation. After 48 hours a record was made of the injury to the foliage caused by the spray material, the extent of feeding, and the number of beetles dead and alive in each cage.

The injury caused by a spray was considered to be "slight" when a few discolored spots appeared, "moderate" when these spots were small but generally distributed, "severe" when the surface of a large proportion of the leaves was injured, and "very severe" when all the foliage was damaged by the spray.

The extent of feeding by the beetles on smartweed foliage is indicated graphically in figure 1. The estimates on feeding are the averages obtained after a careful examination of all the foliage on the plants in the treatment.

[4] FLEMING, W. E. DEVELOPMENT OF A STANDARD CAGE METHOD FOR TESTING THE EFFECTIVENESS OF STOMACH-POISON INSECTICIDES ON THE JAPANESE BEETLE. Jour. Agr. Research 48: 115–130, illus. 1934.
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INSECTICIDAL VALUE

DETERMINATION OF COEFFICIENTS OF EFFECTIVENESS

The average mortality in the cages with the standard insecticide (acid lead arsenate), with each concentration of the different materials under test, and in the starvation cages was determined. The average mortality attributed to poisoning by each concentration of a material was obtained by subtracting the average natural mortality in the starvation cages from the average mortality occurring in the treated cages. The effectiveness of each concentration of a material was expressed as a coefficient of the insecticidal action of the standard insecticide.[5] The coefficients of effectiveness of different concentrations of paris green and its homologues are given in table 2.

TABLE 2.—*Effectiveness of paris green and its homologues as stomach-poison insecticides and as repellents for the Japanese beetle, and their effect on foliage*

Material	Concentration	Coefficient of effectiveness [1]	Extent of injury by spray to foliage	Extent of feeding by beetles on sprayed foliage
	Pounds per 100 gallons			
Copper crotonoarsenite [2]	2	0. 175±0. 113	None	Extensive.
	4	.505± .138	Slight	Moderate.
	8	.807± .151	Moderate	Slight.
	16	.918± .090	Severe	Do.
Copper lauroarsenite [2 3]	2	.283± .092	Slight	Moderate.
	4	.302± .096	Moderate	Do.
	8	.387± .064	Severe	Slight.
Copper oleoarsenite	2	0	None	Defoliation.
	4	0	Very slight	Extensive.
	8	.156± .161	Slight	Moderate.
	16	.439± .179	Moderate	Slight.
	32	.622± .206	Very severe	Very slight.
Copper palmitoarsenite	1	0	None	Defoliation.
	2	0	do	Do.
	4	.458± .101	Slight	Moderate.
	8	.590± .108	do	Slight.
	16	.939± .231	Moderate	Do.
	32	1.097± .243	Very severe	Very slight.
Copper stearoarsenite	1	0	None	Defoliation.
	2	0	do	Do.
	4	0	do	Moderate.
	8	.108± .107	Very slight	Do.
	16	.378± .172	Slight	Slight.
	32	.609± .163	Moderate	Do.
Cottonseed-oil green	2	.427± .031	None	Moderate.
	4	.393± .114	Slight	Do.
	8	.473± .090	Moderate	Do.
	16	.625± .096	do	Slight.
Fish-oil green [3 4]	32	.833± .136	Severe	Very slight.
	4	.680± .065	Slight	Moderate.
	8	.546± .147	Moderate	Do.
Paris green	1	0	None	Defoliation.
	2	.311± .061	do	Moderate.
	4	.679± .065	Slight	Do.
	8	.698± .078	do	Slight.
	16	.753± .107	do	Do.
Paris green and flour in ratio 3 : 2	1	.076± .033	None	Extensive.
	2	.298± .050	do	Moderate.
	4	.359± .062	Very slight	Do.
	8	.633± .067	Moderate	Slight.

See footnotes at end of table.

[5] The standard error of each average and each difference was determined, and the error of the coefficient of effectiveness calculated according to the equation

$$\text{Error of quotient} = \pm\frac{P}{S}\sqrt{\frac{p^2}{P^2}+\frac{s^2}{S^2}}$$

where P is the number of beetles poisoned by the material, S is the number poisoned by the standard insecticide, and p and s are the respective standard errors.

TABLE 2.—*Effectiveness of paris green and its homologues as stomach-poison insecticides and as repellents for the Japanese beetle, and their effect on foliage*—Contd.

Material	Concentration	Coefficient of effectiveness	Extent of injury by spray to foliage	Extent of feeding by beetles on sprayed foliage
	Pounds per 100 gallons			
Paris green and summer oil (summer oil 1 pint to 100 gallons).	1	0	None	Extensive.
	2	0	do	Moderate.
	4	.291± .061	Very slight	Do.
	8	.417± .070	Slight	Slight.
	16	.842± .074	Moderate	Very slight.
	32	1.039± .083	do	Do.
Rapeseed-oil green [1]	2	0	None	Defoliation.
	4	0	do	Do.
	8	.167± .077	Moderate	Moderate.
	16	.094± .072	Severe	Slight.
Soybean-oil green	4	.191± .040	None	Moderate.
	8	.232± .099	Slight	Do.
	16	.420± .101	Moderate	Slight.
	32	.526± .126	Severe	Very slight.
Tung-oil green	2	.136± .075	None	Extensive.
	4	.339± .116	Slight	Moderate.
	8	.562± .133	do	Do.
	16	.830± .089	Moderate	Slight.
	32	.913± .167	Severe	Very slight.

[1] As compared with acid lead arsenate = 1.000±0.060.
[2] The injury was so severe at the highest concentration recorded that further tests were not made.
[3] The homologues of paris green, particularly copper laurcarsenite and fish-oil green, when suspended in water had some coarse particles which clogged the fine nozzle of the spray gun.
[4] It was not possible to determine the values of the coefficients of fish-oil green accurately because of the variability of the data.

RELATION BETWEEN THE COEFFICIENT OF EFFECTIVENESS AND THE CONCENTRATION

When the coefficients of effectiveness of the various materials were plotted against the concentrations, a logarithmic curve was obtained. To determine accurately the position of this line, the constants of the equation of the line were determined mathematically by the method of least squares.[5] It was found that the values obtained from the equation $Y'=a+b \log X$, where Y' is the estimated coefficient of effectiveness, $\log X$ is the logarithm of the concentration, and a and b are constants, came the closest to the determined values of the coefficients. It should be noted that this equation will not give the effectiveness at zero concentration, which, of course, is zero, but there appears to be a close agreement between the estimated and the determined values when the materials were used at the rate of 8 pounds to 100 gallons, at which concentration the effectiveness of the materials was compared. The index of correlation, the index of determination, and the standard error of estimate were then calculated.[6] These values are given in table 3.

There is a high correlation between the coefficient of effectiveness and the concentration of all materials, except in the case of rapeseed-oil green. The index of determination shows what proportion of the variance in the dependent variable can be explained by the concomitant variation in the independent variable. It was found that, with the exception noted, more than 75 percent of the variance in the coefficients of effectiveness could be accounted for by the change in the concentration. Since this leaves only a small percentage of the variance to be accounted for by all other factors, it appears that the concentration was the most important factor associated with the coefficient of effectiveness of these materials.

[5] EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. chs. 7–8. New York and London. 1930.

TABLE 3.—*Statistical measures showing the association between the coefficients of effectiveness of paris green and its homologues and their concentration in the spray*

Material	Index of correlation	Index of determination	Standard error of estimate [1]
		Percent	
Tung-oil green	0.9876	0.9754	0.0501
Copper palmitoarsenite	.9751	.9508	.1020
Copper crotonoarsenite	.9696	.9402	.0816
Paris green and summer oil	.9656	.9324	.1121
Paris green and flour	.9628	.9270	.0619
Soybean-oil green	.9625	.9263	.0429
Copper oleoarsenite	.9455	.8939	.0903
Paris green	.8964	.8036	.1437
Cottonseed-oil green	.8789	.7724	.0865
Copper lauroarsenite	.8737	.7633	.0269
Copper stearoarsenite	.8706	.7579	.1255
Rapeseed-oil green	.5174	.2677	.0693

[1] In terms of the coefficient of effectiveness.

The standard error of estimate may be used as a measure of the consistency of the insecticidal action of a material. Copper lauroarsenite, soybean-oil green, and tung-oil green were the least variable and copper stearoarsenite was the most variable of the homologues of paris green, but the last material was less variable than paris green. When flour or summer oil was mixed with paris green, the variation in the insecticidal results with this material was reduced.

Theoretically, when the linear correlation is high, the averages of several observations for each concentration of a material should lie along the regression line, and these averages should coincide with the estimated values. An examination of the different regression lines showed that the experimentally determined values were arranged about the lines but did not always coincide. It is to be expected in insecticidal tests, even under controlled conditions, that there will be some fluctuation in the determined values. To establish these points more definitely it would be necessary to make many more tests than was possible during the limited period in the summer when the adult beetle is available for experimentation. It is believed that under the circumstances the values for a given concentration obtained from the regression line probably afford a better basis of comparison than the determined values. It was decided, therefore, to use the estimated values of the coefficients of effectiveness at a concentration of 8 pounds to 100 gallons for determining the relative effectiveness of paris green and its various homologues.

As these estimated coefficients of effectiveness are subject to variation, as are other statistical constants, the standard errors of the coefficients were determined by the procedure outlined by Ezekiel.[7] The coefficients were than arranged in order of increasing difference from the coefficient of acid lead arsenate, and the significance of the difference was determined. These data are given in table 4.

[7] EZEKIEL, M. See p. 253 of reference cited in footnote 6.

TABLE 4.—*Comparison of the coefficients of effectiveness of lead arsenate and paris green and its homologues*

Material	Coefficient of effectiveness [1]	Comparison of the coefficients of effectiveness of material indicated with coefficients of less effective materials shown in first column			
		Acid lead arsenate		Copper crotonoarsenite	
		Difference	Difference÷error	Difference	Difference÷error
Acid lead arsenate	1.000±0.060				
Copper crotonoarsenite	.728± .045	0.272±0.075	3.63		
Paris green	.677± .079	.323± .099	3.26	0.051±0.091	0.56
Copper palmitoarsenite	.635± .043	.365± .074	4.93	.093± .062	1.50
Paris green and flour	.601± .052	.399± .079	5.05	.127± .069	1.84
Tung-oil green	.556± .023	.444± .064	6.94	.172± .051	3.37
Cottonseed-oil green	.550± .039	.450± .072	6.25	.178± .060	2.97
Paris green and summer oil	.544± .048	.456± .077	5.92	.184± .066	2.79
Copper lauroarsenite	.376± .024	.624± .065	9.60	.352± .051	6.90
Soybean-oil green	.283± .023	.717± .064	11.20	.445± .051	8.73
Copper stearoarsenite	.244± .053	.756± .080	9.45	.484± .070	6.91
Copper oleoarsenite	.243± .040	.757± .072	10.51	.485± .060	8.08
Rapeseed-oil green	.088± .038	.912± .071	12.85	.640± .059	10.85

Material	Comparison of the coefficients of effectiveness of material indicated with coefficients of less effective materials shown in first column					
	Tung-oil green		Copper lauroarsenite		Soybean-oil green	
	Difference	Difference÷error	Difference	Difference÷error	Difference	Difference÷error
Cottonseed-oil green	0.006±0.045	0.13				
Paris green and summer oil	.012± .053	.23				
Copper lauroarsenite	.180± .034	5.29				
Soybean-oil green	.273± .034	8.03	0.093±0.034	2.74		
Copper stearoarsenite	.312± .058	5.38	.132± .059	2.24	0.039±0.058	0.67
Copper oleoarsenite	.313± .046	6.80	.133± .047	2.83	.040± .046	.87
Rapeseed-oil green	.468± .044	10.64	.288± .045	6.40	.195± .044	4.43

[1] Determined for 8 pounds of material from the regression equation $Y'=a+b \log X$.

It is generally accepted that a value may be considered significant when it is at least two times its standard error. By this criterion of significance, acid lead arsenate was found to be significantly more effective as an insecticide than paris green or any of its homologues. Copper crotonoarsenite, which was about 73 percent as effective as the standard, was not significantly different from copper palmitoarsenite, paris green, and paris green and flour. It was not possible to demonstrate significant differences between tung-oil green, which was about 56 percent as effective as the standard, and cottonseed-oil green, or paris green with summer oil. Copper lauroarsenite, which was about 38 percent as effective as the standard, was significantly less effective than tung-oil green and significantly more effective than soybean-oil green, copper stearoarsenite, copper oleoarsenite, and rapeseed-oil green. Soybean-oil green was not significantly more effective than copper stearoarsenite and copper oleoarsenite. Rapeseed-oil green was significantly less effective than the other materials and was only about 9 percent as effective as acid lead aresenate.

The coefficient of regression measures the slope of the regression line; it shows, in the present case, the average change in the coefficient of effectiveness with each unit increase in the logarithm of the concentration. The variation likely to be present in the coefficient of regression of each material was determined by the procedure outlined by Ezekiel.[8] The coefficients of regression were arranged in order of decreasing magnitude and the significance of the differences between them was determined, as given in table 5.

TABLE 5.—*Significance of difference in change in effectiveness of paris green and its homologues with change in concentration of the spray as shown by their regression coefficients*

Material	Coefficient of regression	Difference between coefficient of regression of indicated material and those of less rapidly changing materials shown in the first column			
		Copper crotonoarsenite		Copper stearoarsenite	
		Difference	Difference÷error	Difference	Difference÷error
Copper crotonoarsenite	0.8408±0.1212				
Copper palmitoarsenite	.8005± .0810	0.0403±0.1458	0.28		
Paris green and summer oil	.7448± .0890	.0960± .1504	.64		
Tung-oil green	.6793± .0537	.1615± .1326	1.22		
Paris green	.6289± .1509	.2119± .1935	1.09		
Paris green and flour	.5753± .0924	.2655± .1524	1.74		
Copper oleoarsenite	.5501± .0949	.2817± .1539	1.83		
Copper stearoarsenite	.4069± .0997	.4339± .1569	2.77		
Soybean-oil green	.3963± .0637	.4445± .1369	3.25	0.0106±0.1183	0.09
Cottonseed-oil green	.3468± .0908	.4940± .1514	3.26	.0601± .1348	.45
Copper lauroarsenite	.1727± .0633	.6681± .1367	4.89	.2342± .1181	1.98
Rapeseed-oil green	.1492± .1039	.6916± .1591	4.35	.2577± .1433	1.80

Copper crotonoarsenite, which increased in effectiveness at the average rate of about 84 percent of the logarithm of the concentration, did not differ significantly in its rate of change from copper palmitoarsenite, tung-oil green, copper oleoarsenite, or paris green, alone or mixed with flour or summer oil. Copper stearoarsenite changed in effectiveness at the rate of about 41 percent of the logarithm of the concentration and did not differ significantly from soybean-oil green, cottonseed-oil green, copper lauroarsenite, and rapeseed-oil green.

EFFECT OF QUANTITY OF RESIDUE ON FOLIAGE

The residues on the foliage were most conspicuous with those materials having a high coefficient of regression. Although no chemical analysis was made of the sprayed foliage to determine the effect of the change in concentration on the quantity of residue, it is apparent that the quantity deposited on the foliage must have increased most rapidly with materials having a high coefficient of regression.

EFFECT OF ARSENIOUS OXIDE CONTENT

It was believed that there might be some correlation between the effectiveness of paris green and its homologues as insecticides and the percentage of the total or water-soluble arsenious oxide in the compounds. As no correlation was found, it is apparent that factors other than the arsenious oxide content are more important in modifying the insecticidal action of these materials.

[8] EZEKIEL, M. See p. 252 of reference cited in footnote 6.

CONCLUSION AS TO INSECTICIDAL EFFECTIVENESS

It appears from these several considerations that copper crotonoarsenite, which was 73 percent as effective as acid lead arsenate when used at a concentration of 8 pounds to 100 gallons, and which increased in effectiveness at the rate of 84 percent of the logarithm of the concentration, was the most promising in this group of materials as an insecticide.

FEEDING BY BEETLES ON SPRAYED FOLIAGE

The Japanese beetle is an intermittent feeder, moving from one part of the foliage to another and making several small punctures, such as those shown in figure 1, *A* and *B*. When the foliage is palatable and several beetles are present, these punctures increase in number and size until all the tissue between the heavy veins of a leaf has been consumed. With a gregarious insect such as the Japanese beetle feeding in this manner, it is not possible to determine accurately the area of a leaf consumed by one beetle, but only to make a general estimate of the extent of feeding.

It was assumed that, for a material to be of value in protecting foliage from injury, the feeding should result in not more than a few small punctures at the end of the test period. This condition was obtained when copper crotonoarsenite, copper lauroarsenite, copper palmitoarsenite, or paris green, alone or in combination with flour or summer oil, was used at the rate of 8 pounds to 100 gallons, but the other arsenites had to be used at the rate of 16 pounds or more to give this degree of protection. It is apparent, however, that factors other than the toxicity of the material deter the beetles from feeding, for copper lauroarsenite, which is significantly less effective than copper crotonoarsenite as an insecticide, was equally as effective in making the foliage unpalatable.

INJURY BY THE MATERIALS TO FOLIAGE

It has been repeatedly observed that injury by spray materials to the foliage occurs more rapidly under conditions of high temperature and humidity, such as prevailed in these tests, than under other conditions. It might be concluded, therefore, that the conditions of these tests are the most exacting in determining the tolerance of foliage to the chemicals.

In each case the materials caused some injury to the foliage at a concentration lower than that which deterred the beetles from feeding. It is believed, therefore, that paris green and its homologues cannot be used in their present form to protect foliage from attack without the possibility of injuring the foliage.

SUMMARY

A study has been made of the relative effectiveness of paris green and its homologues as stomach-poison insecticides against the adult Japanese beetle (*Popillia japonica* Newman). The experiments were conducted under conditions in which the temperature, relative humidity, light, and food were controlled and beetles from the same source were used. The effectiveness of the materials as stomach

poisons was determined by comparison with acid lead arsenate applied to foliage at the rate of 8 pounds to 100 gallons of water and tested under the same conditions, and was expressed as the coefficient of the insecticidal action of the lead arsenate.

It was found that, except in the case of rapeseed-oil green, there was a high correlation between the coefficient of effectiveness of a material and its concentration. In general, 75 percent or more of the variance in the coefficients of effectiveness could be accounted for by the change in the concentration of the materials.

Acid lead arsenate was significantly more effective as an insecticide than paris green or its homologues. Copper crotonoarsenite, which was about 73 percent as effective as lead arsenate, was not significantly different in effectiveness from paris green or copper palmitoarsenite. It was not possible to demonstrate significant differences between the coefficients of effectiveness of tung-oil green and cottonseed-oil green. Copper lauroarsenite was about 38 percent as effective as the standard, and was significantly less effective than tung-oil green and significantly more effective than soybean-oil green, copper stearoarsenite, copper oleoarsenite, and rapeseed-oil green. Soybean-oil green was not significantly more effective than copper stearoarsenite and copper oleoarsenite. Rapeseed-oil green was significantly less effective than the other materials and was only about 9 percent as effective as acid lead arsenate.

Copper crotonoarsenite increased in effectiveness at the average rate of about 84 percent of the logarithm of the concentration and did not differ significantly in its rate of change from copper palmitoarsenite, tung-oil green, copper oleoarsenite, or paris green, alone or mixed with flour or summer oil. Copper stearoarsenite changed in effectiveness at the rate of about 41 percent of the logarithm of the concentration and did not differ significantly from soybean-oil green, cottonseed-oil green, copper lauroarsenite, and rapeseed-oil green.

There was no correlation between total or water-soluble arsenious oxide content and the effectiveness of the materials.

It was necessary to use copper crotonoarsenite, copper lauroarsenite, copper palmitoarsenite, and paris green, alone and in combination with flour or summer oil, at the rate of 8 pounds to 100 gallons to reduce the feeding by 200 beetles to only a few punctures on the foliage. The other materials had to be used at a concentration of 16 pounds or more to obtain this condition. As copper lauroarsenite was significantly less effective than copper crotonoarsenite as an insecticide but equally as effective in making the foliage unpalatable to the beetles, it is apparent that factors other than toxicity deter the beetles from feeding.

As some injury to foliage was caused by paris green and its homologues at lower concentrations than those that deterred the beetles from feeding, it is believed that these materials cannot be safely applied in their present form to control the beetle in the field.

Copper crotonoarsenite was the most promising of the materials studied as an insecticide for control of the Japanese beetle.

DERRIS AS A JAPANESE BEETLE REPELLENT AND INSECTICIDE[1]

By WALTER E. FLEMING, *entomologist*, and FRANCIS E. BAKER, *assistant entomologist, Division of Fruit Insect Investigations, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture*

INTRODUCTION

It has been known for several years that the Japanese beetle (*Popillia japonica* Newm.) does not feed readily on foliage completely covered with lead arsenate, lime, and similar materials (*2*, *3*, *9*, *10*, *13*, *16*, *19*).[2] In 1932 and 1933 (*17*) it was found that spraying with suspensions of derris or rotenone at weekly intervals and after every rain protected foliage from severe injury by the beetle. The use of these sprays has the added advantage that the residue left on the plants is not conspicuous and is probably not harmful to man or animals.

It is difficult, however, to establish definitely the value of a material under field conditions, because of the many variable factors. Some orchards are heavily infested with beetles one season and lightly infested the next. Some orchards are heavily infested early in July and few beetles are present 2 or 3 weeks later; other orchards may not become heavily infested until late in July. The timeliness of the application of a spray is important. When spraying is delayed until beetles have become established on the plants, it is difficult to protect the plants from injury. Many materials that are effective against a light infestation have been found to be inadequate when beetles are very abundant. In view of these variable factors, it was decided to study derris and its components [3] under artificially controlled conditions of heavy infestation where the beetles were stimulated to feed.

DERRIS, ROTENONE, AND DEGUELIN AS CONTACT INSECTICIDES

It has been observed frequently in the field that the application of derris or rotenone sprays to a tree on which beetles are feeding causes large numbers to drop to the ground, and many of these paralyzed beetles subsequently die. A series of laboratory tests was conducted to determine the relative effectiveness of derris and two of its components, rotenone and deguelin, as contact insecticides, as compared with neutral potassium oleate, a material recognized as being effective as a contact insecticide against this insect.

The tests were conducted according to the procedure outlined by Fleming and Baker (*5*). The derris, rotenone, and deguelin were mixed with acetone at the rate of 4 g per 100 cc and added to water so that the concentration ranged from 0.5 to 20 g per liter, which was equivalent to 0.4 to 16 pounds per 100 gallons. These suspensions were kept homogeneous during the treatment by constant stirring.

[1] Received for publication Apr. 9, 1936; issued September 1936.

[2] Reference is made by number (italic) to Literature Cited, p. 206.

[3] The derris, containing 4 percent of rotenone, and the rotenone, 90 percent pure, were purchased on the open market; the deguelin, tephrosin, toxicarol, and derris resin, a material from which all crystalline substances had been removed, were prepared by E. P. Clark, of the Division of Insecticide Investigations.

Neutral potassium oleate soap, which was used as the standard insecticide, was prepared in concentrations ranging from 1.25 to 20 g of anhydrous soap per liter. Five groups, each of 100 beetles, were submerged for 120 seconds in each suspension at a temperature of 80° F. and then placed in cages held at 80° and a relative humidity of 96 to 100 percent for 24 hours to determine the effect of each treatment. Equal numbers of untreated beetles were placed in the cages to determine the natural mortality.

The average survival of 100 beetles treated with each concentration of the materials under test, and with the standard insecticide, and the average survival of the untreated beetles, with their respective standard errors, were determined. Since the mortality of the untreated beetles was assumed to be the natural mortality of the beetles used in the tests, one-half the survival among these beetles was selected as the median point where 50 percent were killed by the insecticidal treatment. The median effective concentration was then calculated according to the equation given by Fleming and Baker (7). The average survival of 100 beetles with each concentration of the materials and with their respective standards, the median effective concentrations, and the coefficients of effectiveness are given in table 1.

TABLE 1.—*Effectiveness of derris, rotenone, and deguelin as contact insecticides against the Japanese beetle as compared with neutral potassium oleate as the standard*

Material	Concentration	Average survival of 100 beetles		Median effective concentration		Coefficient of effectiveness
		Material	Standard	Material	Standard	
	Grams per liter	*Number*	*Number*	*Grams per liter*	*Grams per liter*	
Rotenone	0.00	97±0.83	97±0.83	0.717±0.060	1.651±0.060	2.302±0.210
	.50	72±4.97	----------			
	.75	45±4.30	----------			
	1.00	26±3.05	----------			
	1.25	26±5.68	70±2.99			
	2.50	1± .58	33±2.59			
	5.00	0	17±1.36			
	10.00	0	1± .77			
Deguelin	0.00	95± .55	95± .55	2.537± .137	2.537± .099	1.000± .067
	1.25	74±3.20	81±3.20			
	2.50	48±1.79	48±1.30			
	5.00	15±2.78	15± .97			
	10.00	0	2± .80			
	20.00	0	0			
Derris	0.00	95± .55	95± .55	6.560±2.364	2.537± .099	.387± .140
	1.25	83±2.07	81±3.20			
	2.50	70±3.88	48±1.30			
	5.00	50±3.42	15± .97			
	10.00	42±3.84	2± .80			
	20.00	8±2.12	0			
Derris with equal weight of castor oil.	0.00	97± .83	97± .83	2.968± .274	1.651± .060	.556± .055
	.31	84±1.22	----------			
	.62	66±2.48	----------			
	1.25	64±1.36	70±2.99			
	2.50	56±4.20	33±2.59			
	5.00	16±3.40	17±1.36			
	10.00	0	1± .77			
Derris with 0.125 percent potassium coconut-oil soap	0.00	89±1.22	89±1.22	1.239± .225	2.188± .135	1.766± .339
	1.00	50±4.84	73±3.44			
	2.00	27±5.31	50±3.58			
	3.00	21±2.17	21±2.42			
	4.00	11±2.76	----------			

In the tests in which derris and deguelin were used at the rate of 20 g per liter, half of the liquid was acetone. However, as the survival after treatment with acetone at this concentration was 85 ± 1.50,

it is apparent that the acetone did not appreciably influence the insecticidal results. Statistically there is no significant difference between the coefficients of derris alone and of derris mixed with castor oil, but when derris was added to a 0.125-percent solution of potassium coconut-oil soap, which alone did not kill beetles, the effectiveness was increased to 1.765 times the standard, which was not significantly different from that of rotenone.

These data suggest that if a small quantity of soap is added to the derris spray in the field the effectiveness of the material as a contact insecticide is increased.

DERRIS AND ITS COMPONENTS AS STOMACH-POISON INSECTICIDES AND REPELLENTS

A study was also made of the relative effectiveness of derris and its components as stomach poisons and repellents against the Japanese beetle. The derris was mixed with water at the rate of 250 g to 750 cc of water and passed five times through a colloid mill to obtain a finely divided suspension. The deguelin, rotenone, tephrosin, toxicarol, and derris resin were mixed with acetone at the rate of 2 g to 100 cc. The materials were added to water to obtain sprays containing 1.25, 2.5, 5, 10, 20, and 40 g per liter, which was approximately equivalent to 1, 2, 4, 8, 16, and 32 pounds to 100 gallons. A spray containing 10 g of acid lead arsenate and 2.5 g of light-pressed fish oil per liter was used as a standard insecticide.

The details of the environmental conditions and the procedure for testing stomach-poison insecticides on the Japanese beetle have been described by the authors (*4*, *6*). The method used in applying these materials is outlined in the paper on paris green and its homologues as insecticides against the Japanese beetle (*8*).

The average mortality obtained in cages with the standard insecticide (acid lead arsenate), with each concentration of the various materials under test, and in the starvation cages, together with their respective errors, was determined. The average mortality attributed to poisoning by each concentration of a material was obtained by subtracting the average natural mortality in the starvation cages from the average mortality due to the insecticidal treatments. For this difference the standard error was computed. The effectiveness of each concentration was expressed as a coefficient of the insecticidal action of the standard insecticide by dividing the average number of beetles poisoned by the average number poisoned in the standard.

The coefficients of effectiveness of derris and its components, and the extent of feeding by the beetles on the sprayed foliage, are given in table 2.

It was found that derris and its components, with the possible exception of rotenone, were of little value as stomach-poison insecticides. This confirms the results of previous investigations (*6*) in which it was shown that derris is only a weak stomach poison against the Japanese beetle. There was no injury to the foliage due to these sprays.

The beetles fed to only a limited extent on foliage sprayed with derris, even when the material was used at the rate of 2 pounds to 100 gallons. It is apparent that the repellent action of derris can be attributed to the rotenone and deguelin, as the toxicarol, tephrosin, and the derris resin had little deterrent effect on the insect.

TABLE 2.—*Effectiveness of derris and its components as stomach poisons and repellents against the Japanese beetle as compared with the standard, acid lead arsenate*

Material	Concentration	Coefficient of insecticidal effectiveness	Extent of feeding by the beetle on smartweed foliage.
	Pounds per 100 gallons		
Derris	1	0	Moderate.
	2	0	Light.
	4	0	Very light.
	8	0	Do.
	16	0	Do.
	32	0	Do.
Rotenone	1	0	Do.
	2	0.438±0.044	Do.
	4	.534± .038	Do.
Deguelin	1	0	Light.
	2	0	Do.
	4	0	Very light.
Toxicarol	1	0	Extensive.
	2	0	Do.
	4	0	Moderate.
	8	0	Do.
Tephrosin	1	0	Complete defoliation.
	2	0	Do.
	4	0	Extensive.
	8	0	Do.
Derris resin	1	0	Complete defoliation.
	2	0	Extensive.
	4	0	Moderate.
	8	0	Do.
Acid lead arsenate	8	1.000± .029	Light.

DERRIS AND OTHER MATERIALS AS REPELLENTS WHEN APPLIED TO HEAVILY INFESTED APPLES

Apples and peaches that ripen during the period when the beetles are numerous are not easily protected by the application of repellent sprays, because the attractiveness of the fruit overcomes to a large extent the effect of the spray. A study was made of the effectiveness, under controlled laboratory conditions, of various repellent sprays that had been found to be effective under favorable conditions in the field.

Twigs of apple of the variety Yellow Transparent were placed in 3-inch earthen pots of soil. The surface of the soil was covered with plaster of paris to hold the twigs firmly in position. Various sprays of lime, acid lead arsenate, rotenone, and derris, in most cases in combination with a sticker, were applied to the twigs under a pressure of 20 pounds per square inch. During this application apples that had been cut in half to make them more attractive to the beetles were fastened on wires and held in the different sprays until thoroughly coated. When the spray deposit was dry on the fruit and foliage, two half apples were suspended on each twig and placed in the special glass cages under controlled conditions. Three twigs were placed in each cage and five cages were used for each treatment. Then 200 freshly collected beetles, which had been starved for 6 hours, were liberated in each cage. After 48 hours a record was made of the extent of feeding by the beetles. A summary of these results is given in table 3.

TABLE 3.—*Effectiveness of derris, rotenone, hydrated lime, and acid lead arsenate as repellents to the Japanese beetle when applied to Yellow Transparent apples*

Material and sticker	Concentration		Extent of feeding by beetles—	
	Material	Sticker	On the fruit	On the foliage
	Pounds per 100 gallons	*Pounds per 100 gallons*		
No treatment			Very extensive	Very extensive.
Derris with light-pressed fish oil	3	0	None	None.
	3	1	do	Do.
	4	2	do	Do.
	8	2	do	Do.
Rotenone	4	0	do	Do.
	8	0	do	Do.
Hydrated lime	32	0	Extensive	Moderate.
Hydrated lime with light-pressed fish oil	20	1	do	Do.
Hydrated lime with flour	20	3	do	Do.
Hydrated lime with aluminum sulphate	20	3	do	Do.
Acid lead arsenate with light-pressed fish oil	6	1.5	Slight	None.
	8	2	do	Do.
Acid lead arsenate with flour	6	4	do	Do.

It was found under these artificial conditions of heavy infestation, where the beetles were stimulated to feed and had no choice between sprayed and unsprayed plants, that the application of lime at the rate of 32 pounds to 100 gallons, or mixed with fish oil, flour, or aluminum sulphate and used at the rate of 20 pounds to 100 gallons, did not protect the fruit from injury, although the feeding on the foliage was decreased. Acid lead arsenate, mixed with fish oil or with flour, protected the foliage but did not prevent the beetles from feeding to a slight extent on the fruit. Derris, alone or with fish oil, and rotenone afforded complete protection to the fruit and foliage.

The effect of lime and of acid lead arsenate under these artificial conditions was inferior to that usually obtained under conditions of moderate infestation in the field, which might be attributed to the fact that the beetles had no choice between sprayed and unsprayed plants. The results with derris and with rotenone were superior to those obtained in the field, probably because under the artificial conditions the materials were not subject to decomposition by sunlight or to mechanical loss by wind and rain.

EFFECT OF EXPOSURE TO ULTRAVIOLET LIGHT ON EFFECTIVENESS OF DERRIS AND ROTENONE

There are several statements in the literature that derris and rotenone are altered by exposure to light. Davidson and Jones (*1*), Jones and Haller (*15*), Gersdorff (*11*), and Tattersfield and Roach (*18*) have demonstrated that derris and rotenone in solution lose toxicity. Tests by Newcomer, Hough, and Lathrop against the codling moth and by Ginsburg against the silkworm [4] indicate that spray deposits lose much of their effectiveness after several days' exposure to sunlight. Jones, Gersdorff, Gooden, Campbell, and Sullivan (*14*) have shown that derris and rotenone are rapidly decomposed by direct sunlight, with a resulting decrease in toxicity to insects. It was observed during the seasons of 1932, 1933, and 1934 that deposits of derris and rotenone

[4] CAMPBELL, F. L. REVIEW OF INFORMATION ON THE INSECTICIDAL VALUE OF ROTENONE. U. S. Dept. Agr., Bur. Ent. E-298, 28 pp. 1932. [Mimeographed.]

on the foliage lost much of their effectiveness as repellents to the Japanese beetle 3 or 4 days after application, but it was not known whether this loss was due to weathering or to a chemical change in the material.

Suspensions of derris, rotenone, and deguelin were applied to the foliage of smartweed (*Polygonum pennsylvanicum*). The sprayed plants were then set on a slowly rotating table and passed under the hood of a horizontal quartz mercury-vapor arc, placed 18 inches above the plants in such a manner that the light was directed on the foliage. The radiation of this lamp extended from the 10,140 angstrom line in the infrared to the region of 1,850 angstrom in the ultraviolet. Groups of plants were exposed for periods of 1, 12, and 24 hours and were then placed in cages with beetles. Unfortunately, the exposure of the plants to the ultraviolet light caused a loss of turgidity and browning of the foliage; so it was impossible to determine definitely the reaction of the beetles.

In view of the adverse effect of ultraviolet light on the plants, it was decided to expose derris and rotenone to the light before applying it to the foliage. Each material was made into a thin paste with acetone (20 g of material to 100 cc of acetone) and painted on glass plates, a margin being left around the edge to prevent loss in handling. The materials were then exposed for periods of 1 and 24 hours. At the end of the exposure, the deposits were reground in a mortar and applied to smartweed foliage. The reaction of the beetles to the plants sprayed with these materials is given in table 4.

TABLE 4.—*Effect of exposure to ultraviolet light on derris and rotenone as repellents and stomach poisons against the Japanese beetle*

Material	Concentration	Exposure to ultraviolet light	Coefficient of insecticidal effectiveness	Extent of feeding on smartweed foliage
	Pounds per 100 gallons	*Hours*		
Derris	4	None	0	Very light.
		1	0	Moderate.
		24	0	Do.
	8	None	0	Very light.
		1	0	Light.
		24	0	Do.
	16	None	0	Very light.
		1	0	Light.
		24	0	Do.
Rotenone	4	None	0.534±0.038	Very light.
		1	0	Do.
		24	0	Light.
	8	None	.477± .040	Very light.
		1	0	Do.
		24	0	Light.
	16	None	.584± .056	Very light.
		1	.230± .046	Do.
		24	0	Light.

It was not possible to detect the change in effectiveness of derris as a stomach poison after exposure to ultraviolet light, because of the low toxic value of the material. Rotenone, on the other hand, was definitely decreased in toxicity. When used at the rate of even 16 pounds to 100 gallons, it lost all its toxic value as a stomach poison after exposure to ultraviolet light for 24 hours.

The value of these materials as repellents was only slightly reduced by exposure to ultraviolet light for 24 hours, indicating that their repellent effect is not destroyed so rapidly as is their toxicity. It appears from these experiments, however, that some of the loss in effectiveness of the derris and rotenone sprays can be attributed to the decomposition of the materials in sunlight. There is no satisfactory method at present for preventing the decomposition of these materials.

EFFECT ON ITS EFFECTIVENESS OF ADDING MATERIALS TO DERRIS

A study was made of the effect of adding different materials to derris on its effectiveness as a stomach poison and as a repellent for the Japanese beetle. The results of these experiments are summarized in table 5.

TABLE 5.—*Effect of the addition of other materials to derris on its effectiveness as a stomach-poison insecticide and as a repellent to the Japanese beetle*

Material added to derris	Concentration		Coefficient of effectiveness	Extent of feeding on smartweed foliage	Remarks
	Material	Derris			
	Pounds per 100 gallons	*Pounds per 100 gallons*			
None	0	2	0	Light	
		4	0	Very light	
		8	0	do	
		16	0	do	
		32	0	do	
Light-pressed fish oil	2	4	0	Light	
		8	0	Very light	
		16	0	do	
		32	0	do	
Molasses	8	0	0	Defoliation	Foliage sticky.
		4	0	Light	
		8	0	Very light	
		16	0	do	
		32	0	do	
	32	0	0	Defoliation	Foliage very sticky.
		4	0	Light	
		8	0	Very light	
		16	0	do	
		32	0	do	
Phenothiazine	2	0	0	Defoliation	Slight narcotic effect on beetles.
		2	0	Moderate	
		4	0	Light	
		8	0	do	
		16	0	Very light	
	4	0	0	Defoliation	Do.
		2	0	Moderate	
		4	0	Light	
		8	0	do	
	8	0	0	Extensive	Definite narcotic effect.
	16	0	0	Moderate	
	32	0	0	do	
Sodium silicate	8	0	0	Defoliation	
		2	0	Moderate	Derris suspension became red.
		4	0	do	
		8	0	Light	
	16	0	0	Defoliation	
		2	0	Extensive	Do.
		4	0	do	
		8	0	Moderate	
Tannic acid	4	0	0	Defoliation	
		4	0	Light	
		8	0	do	
	8	0	0	Defoliation	
		4	0	Very light	
		8	0	do	

It was found that the addition of fish oil to derris did not modify the effectiveness of the material as a stomach poison or as a repellent. When molasses was sprayed on the foliage at the rate of 8 pounds to 100 gallons, the plants were very attractive to the beetles and all the foliage was consumed in 24 hours; when the molasses was used at the rate of 32 pounds to 100 gallons, the beetles were repelled somewhat by the sticky surface of the leaves but defoliated the plants. When derris was mixed with molasses, beetles went readily to the foliage but fed only to a limited extent. Phenothiazine appeared to have a narcotic effect on the beetles, but it was not repellent or toxic. Phenothiazine also appeared to decrease the value of the derris as a repellent.

Sodium silicate solution, containing 6.4 percent Na_2O and 24.7 percent SiO_2, and having a pH of approximately 10.5, when added to derris to increase its adhesiveness, decomposed the derris and destroyed its value as a repellent. Tannic acid, under some conditions in the field, appeared to deter the beetles from feeding. Under these artificial conditions tannic acid was of no value in protecting the foliage from attack. The addition of tannic acid to derris sprays did not appreciably modify their effectiveness.

EFFECT OF VARIOUS STICKERS ON THE REPELLENCY OF DERRIS BEFORE AND AFTER WASHING

It has been observed during the past 2 years that if a rain occurred a few hours after the application of derris or rotenone in the field most of the deposit was removed and the plants were subject to attack by the beetles. It was believed that this mechanical loss by the action of rain, and possibly of wind and dew, was one of the most important factors, if not the most important factor, limiting the effectiveness of derris sprays in this vicinity. The following materials were therefore tried as stickers to increase the adhesiveness of derris to foliage: Fish oil, bone glue, bone glue with triethanolamine, emulsified paraffin, rosin and castor oil, castor oil and glue emulsified with oleic acid and triethanolamine, and tannic acid. The fish oil and tannic acid were added directly to the derris; the paraffin, with a melting point of about 110° F., was emulsified with oleic acid and triethanolamine according to the procedure outlined by Ginsburg (*12*); the rosin and castor oil were heated on a water bath until the rosin was in solution; the mixture of castor oil, glue, oleic acid, and triethanolamine was added to an equal weight of water and boiled until homogeneous.

These materials were added in different proportions to the derris suspensions and sprayed on smartweed plants. After the spray deposit had dried, half of the sprayed plants were subjected for 10 minutes to a coarse spray of water under 20 pounds' pressure. When the plants were dry, they were placed in cages with 200 beetles under the controlled conditions previously described. The effect of the different stickers on derris, before and after washing, is given in table 6.

It was apparent that derris without a sticker is readily removed from foliage by washing, as beetles that refused to feed on the unwashed foliage consumed all the foliage after it had been washed. The addition of fish oil, bone glue, and tannic acid did not appear to increase the adhesiveness of the deposit. When triethanolamine was

TABLE 6.—*Effect of various stickers on derris, used at the rate of 4 pounds to 100 gallons, as a repellent to the Japanese beetle, before and after washing the foliage with water*

Stickers added to derris	Concentration of stickers [1]	Extent of feeding on smartweed foliage—	
		Unwashed	Washed
	Pounds per 100 gallons		
None	0	Very light	Defoliation.
Light-pressed fish oil	2	do	Do.
Bone glue	4	do	Do.
	8	do	Extensive.
Bone glue and triethanolamine	2–2	Moderate	Defoliation.
	4–4	Extensive	Do.
	8–8	do	Do.
Rosin and castor oil	1–1	Light	Extensive.
	2–2	do	Do.
	4–4	do	Moderate.
Castor oil, oleic acid, bone glue, and triethanolamine.	1.27–0.12–0.30–0.30	do	Do.
	1.38–0.12–0.20–0.30	do	Do.
	1.48–0.12–0.10–0.30	do	Do.
Paraffin, oleic acid, and triethanolamine	0.80–0.08–0.03	do	Extensive.
	4–0.40–0.15	do	Moderate.
	8–0.8–0.3	do	Do.
	16–1.6–0.6	do	Do.
	40–4–1.6	do	Light.
Tannic acid	4	do	Extensive.
	8	do	Do.

[1] Where combination stickers are used, concentrations are given in order named.

added to the derris and bone-glue mixture, the extent of feeding on unwashed foliage increased with increments of triethanolamine, indicating that the addition of this material was decomposing the derris.

The addition of rosin and castor oil, or of castor oil, oleic acid, bone glue, and triethanolamine, to the derris suspensions did not seem to decompose the derris and appeared to reduce the loss of the material from the foliage during the washing operation. When paraffin was emulsified and used at the concentration of 40 pounds to 100 gallons, the loss of derris appeared to be prevented. In this case the leaves were entirely covered with a waxy deposit, which was resistant to water. The use of paraffin in this concentration is impractical and is likely to injure the foliage. When smaller quantities of paraffin were added to the derris, it was apparent that some of the material was removed by the washing.

It has been demonstrated that derris is readily removed from the foliage by washing, and that some sticker is needed to make the spray satisfactory in the field. However, the results with the materials tested during the summer of 1934 showed that none of these materials was satisfactory and indicate the necessity for further experimentation to develop a suitable sticking agent for derris.

SUMMARY

A study was made of derris and its components under artificially controlled conditions to obtain further information on the value of these materials for the control of the Japanese beetle.

Rotenone was found to be 2.3 times as effective as neutral potassium oleate as a contact insecticide, deguelin was equally as effective as the soap, and derris was 0.387 times as effective. When an equal weight of castor oil was added to the derris, the coefficient of effectiveness was increased to 0.556. When added to a 0.125-percent solution

of coconut-oil soap, which alone did not kill beetles, the effectiveness of derris was increased to 1.765 times that of potassium oleate. These data indicate that if a small quantity of soap is added to the derris spray in the field the effectiveness of the material as a contact insecticide may be greatly increased.

Derris and its components, with the possible exception of rotenone, were found to be of little value as stomach-poison insecticides against this insect. Rotenone when used at the rate of 4 pounds to 100 gallons was 53.4 percent as effective as acid lead arsenate used at the rate of 8 pounds to 100 gallons of water.

Beetles fed only slightly on derris when used at the rate of 2 pounds to 100 gallons. It is apparent that the repellent action of derris can be attributed to its rotenone and deguelin content. Toxicarol, tephrosin, and the derris resin from which all crystalline substances had been removed had little deterrent effect on the insect.

Under artificial conditions of heavy infestation, where the beetles were stimulated to feed and had no choice between sprayed and unsprayed foliage, derris and rotenone afforded complete protection to the fruit and foliage of Yellow Transparent apples, being superior to acid lead arsenate and the lime sprays, which have given satisfactory results under favorable field conditions.

Exposure to ultraviolet light definitely reduced the value of rotenone as a stomach poison against the Japanese beetle. It was not possible to detect the change in effectiveness of derris because of the low toxic value of the material. The value of derris and rotenone as repellents was only slightly reduced by exposure to ultraviolet light for 24 hours, indicating that the repellent effect is not destroyed so rapidly as the toxicity.

Fish oil and tannic acid did not modify the effectiveness of derris as a stomach-poison insecticide or as a repellent. Sodium silicate decomposed the derris and destroyed its value as a repellent.

Derris is readily washed from foliage by water. It is believed that this mechanical loss by the action of rain, and possibly dew, is one of the most important factors limiting the effectiveness of derris sprays for control of the Japanese beetle. Several stickers were tested, but none of them proved satisfactory.

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THE AMMONIATION OF WASTE SULPHITE LIQUOR AND ITS POSSIBLE UTILIZATION AS A FERTILIZER MATERIAL [1]

By M. PHILLIPS, *senior chemist*, M. J. GOSS, *associate chemist, Industrial Farm Products Research Division, Bureau of Chemistry and Soils;* B. E. BROWN, *senior biochemist*, and F. R. REID, *assistant biochemist, Division of Soil Fertility Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Waste sulphite liquor is a byproduct or waste material produced in connection with the preparation of wood pulp by the sulphite process. When wood and similar lignified materials are heated with a solution of sulphurous acid and acid sulphites, the lignin is converted into water-soluble lignin sulphonic acids, leaving the cellulose in a more or less pure condition. The solution containing the lignin sulphonic acids, various carbohydrates, organic acids, and excess of sulphurous acid and bisulphites is generally discarded. In this country alone, approximately 1,500,000 tons of lignin are annually discharged from the various mills producing wood pulp.

Waste sulphite liquor has been the subject of numerous investigations, and there is at present an extensive chemical and patent literature dealing with the utilization or possible utilization of this industrial waste material. As yet, however, no method has been developed for the economic utilization of waste sulphite liquor, although attempts have been made to use it in the preparation of dyes, adhesives, and binding, sizing, and tanning materials, and as a fertilizer.

It is now believed by many agricultural chemists that humus or soil organic matter, which greatly improves the texture and moisture-holding capacity of the soil, has been derived to a considerable extent from lignin. The fact that even under the most favorable conditions, the rate of decomposition of lignin by soil micro-organisms is much less than that of the other major plant constituents, has been advanced as an argument in favor of the view that much of the soil humus was formed from lignin. Lignin constitutes by far the largest percentage of the organic matter of waste sulphite liquor. In view of the fact that there is at present a demand for nitrogenous organic fertilizer materials, largely because the nitrogen contained therein becomes available to the plant gradually, it seemed worth while to attempt to introduce nitrogen into lignin and possibly into the other organic components of the waste sulphite liquor and thus obtain a nitrogenous product having the nitrogen chemically bound to the organic material. The results of a preliminary investigation of the ammoniation of waste sulphite liquor were presented in a previous publication (*9*).[2] In the present paper results are presented on the ammoniation of waste sulphite liquor under various experimental conditions and on the composition of the products thus obtained,

[1] Received for publication Mar. 31, 1936; issued September 1936.
[2] Reference is made by number (italic) to Literature Cited, p. 224.

together with some data on the ammonification and nitrification of these products by soil micro-organisms and their evaluation as fertilizer materials by vegetative greenhouse experiments.

REVIEW OF LITERATURE

Because of its relatively high content of sulphur and organic matter, various attempts have been made to utilize waste sulphite liquor as a fertilizer either by itself or in combination with other materials rich in phosphorus, potash, or nitrogen. The percentage of phosphorus, potash, and nitrogen in sulphite liquor itself is practically nil. A review of the older literature on the utilization of waste sulphite liquor as a fertilizer material has been given by Johnsen and Hovey (*7*), and need not be repeated here. Among the more recent publications on the utilization of waste sulphite liquor as a fertilizer may be mentioned those of Bokorny (*2*, *3*) and Görbing (*5*). Bokorny applied to the soil some neutralized waste sulphite liquor from which the sugars had been removed by fermentation with yeast, which had then been concentrated to one-fifth of its original volume, and obtained increased yields of barley, beans, peas, wheat, and potatoes. Görbing, however, failed to confirm the findings of Bokorny.

Feustel and Byers (*4*) heated lignin from corncobs, and also waste sulphite liquor, with aqueous ammonia for 3 hours at 150° C. The percentage of nitrogen combined with the lignin and waste sulphite liquor amounted to 2.37 and 5.06 percent, respectively.

Scholl and Davis (*11*) heated the residue from waste sulphite liquor for 24 hours with liquid ammonia at 125° C. and obtained a product which contained 4.58 percent of nitrogen. No data on the availability of the nitrogen in the ammoniated product as determined by chemical methods and by greenhouse experiments were presented.

Pinck, Howard, and Hilbert (*10*), by treating alkali lignin from corncobs with liquid ammonia for 24 hours at 180° C., obtained a product which contained 7 percent of nitrogen. They determined the various groups of nitrogenous complexes in their product but presented no data on the availability to plants of the nitrogen in their ammoniated lignin.

MATERIAL AND APPARATUS

All the ammoniation experiments described in this paper were conducted on a batch of sulphite liquor which had been obtained from a paper mill.[3] A portion of this was evaporated to dryness, dried at 105° C., and analyzed by the standard methods of the Association of Official Agricultural Chemists (*1*). The following results were obtained: Total P_2O_5, none; total N, 0.04 percent; total K_2O, 0.15 percent.

For the first group of experiments, a portion of the waste sulphite liquor was neutralized with ammonia, evaporated to dryness, and dried at 105° C. The total nitrogen content of this as determined by the Kjeldahl-Gunning-Arnold method (*1*) amounted to 2.6 percent.

The second group was conducted with material prepared as follows: Ammonia gas was passed into the sulphite liquor until the desired

[3] The sulphite liquor used in these experiments was kindly supplied by Brown Co., of Berlin, N. H.

concentration of ammonia was obtained, and this solution was then heated in the autoclave. Each solution thus prepared was analyzed for total NH_3 and free NH_3. The former was determined by accurately weighing out in a weighing bottle 4 to 5 g of the solution, and making up to volume in a volumetric flask. An aliquot of this was measured into a Kjeldahl flask, 200 cc of distilled water and 10 cc of 50-percent sodium hydroxide were added, and this solution was distilled into a definite volume of a standard acid solution. The free ammonia was determined in a similar manner, except that no sodium hydroxide solution was added to the Kjeldahl flask containing the solution to be analyzed.

The apparatus used in all the ammoniation experiments consisted of two steel bombs of approximately 500-cc capacity. These bombs were fastened to a carrier and revolved in an oil bath provided with thermostatic control. A full description of the apparatus has been given elsewhere (*6*, *8*).

EXPERIMENTAL PROCEDURE

The experimental procedure for the first group of experiments was as follows: Into each bomb 50 g of the dry material, which had been neutralized with ammonia, and the requisite quantity of aqueous ammonia (28 percent NH_3) were placed. The bombs were rotated in the oil bath maintained at a definite temperature for a given period. In each experiment, the reaction products from the two bombs were combined, evaporated to dryness on the steam bath, and dried at 105° C. The product was analyzed for total nitrogen, ammoniacal nitrogen, water-soluble organic nitrogen, and water-insoluble organic nitrogen soluble in neutral permanganate. The analytical methods of the Association of Official Agricultural Chemists (*1*) were employed.[4]

For the second group of experiments 300 g of the sulphite liquor, containing the desired concentration of ammonia, was placed in each bomb and heated for a definite period in the oil bath maintained at a definite temperature. The reaction products from the two bombs were combined, evaporated to dryness on the steam bath, and dried at 105° C. The products obtained were analyzed for the various forms of nitrogen, as already described for the first group.

RESULTS

AMMONIATION OF WASTE SULPHITE LIQUOR AND ITS NEUTRALIZED DRY RESIDUE

Table 1 shows that at a temperature of 200° C. and at a ratio of 600 cc of aqueous ammonia (28 percent NH_3) to 100 g of the dry neutralized residue from waste sulphite liquor the percentage of total nitrogen increased with increase in the period of heating, whereas the percentage of ammoniacal nitrogen decreased. The percentage of water-soluble organic nitrogen expressed as percentage of the total nitrogen decreased as the heating period increased. The percentage of nitrogen of the water-insoluble organic nitrogen soluble in neutral permanganate was somewhat irregular, ranging from 83.6 to 91.3 percent.

[4] The samples from the ammoniation experiments 5, 11, 12, and 18 were analyzed in the laboratory of the Division of Soil Fertility Investigations, Bureau of Plant Industry, under the supervision of E. C. Shorey for ammoniacal nitrogen, water-soluble organic nitrogen, and water-insoluble organic nitrogen soluble in neutral permanganate solution.

TABLE 1.—*Analysis of dry product after ammoniation of solids of waste sulphite liquor with 28-percent aqueous ammonia at 200° and at 220° C., with various periods of heating*

[100 g of dry neutralized residue of waste sulphite liquor and 600 cc of aqueous ammonia (28 percent NH_3) were used for each experiment]

Experiment no.	Time of heating	Total nitrogen	Ammoniacal nitrogen	Water-soluble organic nitrogen		Water-insoluble organic nitrogen			
				In sample	Of total nitrogen	In sample	Of total nitrogen	Soluble in neutral permanganate	
								In sample	Of water-insoluble nitrogen
	Hours	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
200° C.									
1	4	7.30	1.23	6.38	87.4	0.92	12.6	0.82	89.1
2	8	8.32	.36	3.32	39.9	5.00	60.1	4.28	85.6
3	12	8.83	.20	3.32	37.6	5.51	62.4	4.61	83.6
4	16	8.56	.22	3.08	36.0	5.48	64.0	4.76	86.9
5	20	9.07	.19	2.86	31.5	6.21	68.5	5.67	91.3
220° C.									
6	4	8.82	0.33	3.04	34.5	5.78	65.5	5.01	86.7
7	8	9.56	.20	3.52	36.8	6.04	63.2	5.22	86.4
8	12	9.95	.13	3.83	38.5	6.12	61.5	5.31	86.8
9	16	9.92	.15	3.68	37.1	6.24	62.9	5.38	86.2
10	20	10.55	.09	3.65	34.6	6.90	65.4	5.59	81.0
11	30	10.14	.05	3.44	33.9	6.70	66.1	4.16	62.1

When the ammoniation was carried out at a higher temperature, namely, at 220° C., the percentage of total nitrogen in the product increased as the heating period increased, reaching a maximum at a heating period of 20 hours, after which it declined slightly. For the most part, the percentage of ammoniacal nitrogen decreased in a regular manner as the heating period increased. The percentage of water-soluble organic nitrogen expressed as percentage of the total nitrogen showed very little variation with increase in the heating period. The percentage of nitrogen soluble in neutral permanganate expressed as percentage of the water-insoluble nitrogen was affected only to a slight extent when the heating period ranged from 4 to 16 hours. However, the products obtained by heating for 20 to 30 hours did show substantial decreases in the percentage of water-insoluble nitrogen soluble in neutral permanganate.

It will be observed that at 220° C. the ammoniation proceeded much more rapidly than at 200°. At the temperature of 220° and at a heating period of only 4 hours a product was obtained which contained the same percentage of total nitrogen as the material from experiment 3, where the heating period was 12 hours. With the exception of experiment 1, the percentages of water-soluble organic nitrogen in the products from the two series of experiments were of the same general order of magnitude. In the series of experiments conducted at 220° the percentage of water-insoluble nitrogen soluble in neutral permanganate decreased substantially when the reaction mixture was heated for 20 or 30 hours.

In table 2 are presented results of two series of experiments which were conducted for the purpose of ascertaining the optimum ratio of

ammonia to the neutralized sulphite liquor residue. The experiments were carried out at 200° and 220° C., and the optimum reaction period for total nitrogen previously determined, namely, 20 hours (table 1), was employed.

TABLE 2.—*Analysis of dry product after ammoniation of solids of waste sulphite liquor with various quantities of 28-percent aqueous ammonia, at 200° and at 220° C.*

[100 g of dry neutralized residue of waste sulphite liquor were used for each experiment. Time of heating was 20 hours]

Experiment no.	Aqueous ammonia added	Total nitrogen	Ammoniacal nitrogen	Water-soluble organic nitrogen		Water-insoluble organic nitrogen			
				In sample	Of total nitrogen	In sample	Of total nitrogen	Soluble in neutral permanganate	
								In sample	Of water-insoluble nitrogen
	Cc	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
200° C.									
12	500	9.09	0.12	3.69	40.6	5.40	59.4	4.16	77.0
13	400	8.96	.33	3.15	35.2	5.81	64.8	5.02	86.4
14	300	8.67	.24	2.82	32.5	5.85	67.5	4.83	82.6
15	200	8.51	.11	2.39	28.1	6.12	71.9	4.22	68.9
16	100	7.92	.06	1.81	22.9	6.11	77.1	4.12	67.4
220° C.									
17	500	9.88	0.07	3.09	31.3	6.79	68.7	5.22	76.9
18	400	10.00	.09	3.07	30.7	6.93	69.3	4.93	71.1
19	300	9.83	.11	3.23	32.9	6.60	67.1	4.32	65.5
20	200	9.53	.07	2.57	27.0	6.96	73.0	3.90	56.0
21	100	8.47	.05	1.48	17.5	6.99	82.5	3.33	47.6

Table 2 shows that at 200° C. and with a ratio of only 400 to 500 cc of aqueous ammonia to 100 g of neutralized sulphite liquor residue, an ammoniated product was obtained which contained practically the same percentage of total nitrogen as when 600 cc of 28-percent ammonia was used (experiment 5, table 1). The percentage of water-soluble organic nitrogen in the product decreased with the decrease of the ratio of aqueous ammonia to the dry residue of waste sulphite liquor. The nitrogen soluble in neutral permanganate, expressed as percentage of the water-insoluble nitrogen, declined considerably when the ratio of aqueous ammonia to the neutralized residue of waste sulphite liquor was 1:1 or 2:1.

In the experiments conducted at 220° C. the percentage of total nitrogen in the product decreased with the decrease in the ratio of ammonia to the sulphite liquor residue. When 600 cc of aqueous ammonia was used the percentage of total nitrogen in the product amounted to 10.55 (experiment 10, table 1), while when only 100 cc was used the percentage of nitrogen was 8.47. The percentage of water-soluble organic nitrogen as well as the percentage of water-insoluble nitrogen soluble in neutral permanganate decreased decidedly when lower ratios of ammonia with respect to the residue of waste sulphite liquor were used.

In table 3 and in the following tables data are presented on the direct ammoniation of waste sulphite liquor. Instead of ammoniating the neutralized residue of waste sulphite liquor, ammonia gas

was passed into the raw liquor itself until the desired concentration of ammonia was attained, and the solution thus obtained was then heated under pressure in an autoclave under definite conditions of temperature and time of heating.

TABLE 3.—*Analysis of dry product after ammoniation of waste sulphite liquor with ammonia gas at various temperatures and with various periods of heating*

[300 g of sulphite liquor was used]

Experiment no.	Time of heating	Concentration of ammonia		Total nitrogen	Ammoniacal nitrogen	Water-soluble organic nitrogen		Water-insoluble organic nitrogen			
		Total	Free			In sample	Of total	In sample	Of total	Soluble in neutral permanganate	
										In sample	Of water-insoluble nitrogen
	Hours	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
220° C.											
22	10	12.0	11.7	9.42	0.11	2.55	27.1	6.87	72.9	6.10	88.8
23	15	12.0	11.7	9.77	.12	2.49	25.5	7.28	74.5	6.23	85.6
24	20	12.0	11.7	9.30	.14	2.43	26.1	6.87	73.9	5.59	81.4
25	25	12.0	11.7	9.96	.10	2.65	26.6	7.31	73.4	6.19	84.7
26	10	14.9	14.5	9.96	.18	2.19	22.0	7.77	78.0	6.14	79.0
27	15	14.9	14.5	9.70	.10	2.30	23.7	7.40	76.3	5.89	79.6
28	20	14.9	14.5	10.15	.08	1.82	17.9	8.33	82.1	6.82	81.9
29	25	14.9	14.5	10.27	.10	1.99	19.4	8.28	80.6	6.79	82.0
30	10	27.0	26.6	10.18	.08	2.94	28.9	7.24	71.1	6.05	83.6
31	15	27.0	26.6	10.43	.07	3.33	31.9	7.10	68.1	5.92	83.4
32	20	27.0	26.6	11.15	.07	3.19	28.6	7.96	71.4	6.59	82.8
33	25	27.0	26.6	10.58	.04	2.86	27.0	7.72	73.0	6.26	81.1
200° C.											
34	10	11.4	11.1	7.70	0.56	3.20	41.6	4.50	58.4	3.89	86.4
35	15	11.4	11.1	8.21	.40	2.66	32.4	5.55	67.6	4.78	86.1
36	20	11.4	11.1	8.21	.41	2.57	31.3	5.64	68.7	4.99	88.5
37	25	11.4	11.1	8.49	.17	2.45	28.9	6.04	71.1	4.40	72.8
38	10	14.6	14.2	8.46	.59	2.78	32.9	5.68	67.1	5.03	88.6
39	15	14.6	14.2	8.72	.28	2.47	28.3	6.25	71.7	5.39	86.2
40	20	14.6	14.2	9.67	.13	2.71	28.0	6.96	72.0	6.19	88.9
41	25	14.6	14.2	9.15	.18	2.72	29.7	6.43	70.3	5.52	85.8
42	10	17.9	17.8	7.78	.62	3.20	41.1	4.58	58.9	4.01	87.6
43	15	17.9	17.8	7.92	.61	2.64	33.3	5.28	66.7	4.25	80.5
44	20	17.9	17.8	8.43	.43	2.78	33.0	5.65	67.0	4.31	76.3
45	25	17.9	17.8	8.53	.35	2.80	32.8	5.73	67.2	4.39	76.6
46	10	25.6	25.4	8.98	.48	3.05	34.0	5.93	66.0	5.09	85.8
47	15	25.6	25.4	9.33	.32	3.17	34.0	6.16	66.0	5.08	82.5
48	20	25.6	25.4	9.47	.38	3.51	37.1	5.96	62.9	4.97	83.4
49	25	25.6	25.4	9.13	.19	2.78	30.4	6.35	69.6	5.67	89.3
180° C.											
50	10	11.4	11.1	7.00	1.60	5.63	80.4	1.37	19.6	1.28	93.4
51	15	11.4	11.1	7.51	1.34	4.60	61.3	2.91	38.7	2.48	85.2
52	20	11.4	11.1	7.38	.86	3.29	44.6	4.09	55.4	3.60	88.0
53	25	11.4	11.1	7.49	.82	3.56	47.5	3.93	52.5	3.29	83.7
54	10	14.6	14.2	6.45	1.87	5.75	89.1	.70	10.9	.65	92.9
55	15	14.6	14.2	7.41	1.55	5.62	75.8	1.79	24.2	1.71	95.5
56	20	14.6	14.2	7.27	1.48	4.53	62.3	2.74	37.7	2.45	89.4
57	25	14.6	14.2	7.76	1.43	4.56	58.8	3.20	41.2	2.82	88.1
58	10	17.9	17.8	7.33	1.92	6.34	86.5	.99	13.5	.93	93.9
59	15	17.9	17.8	7.10	1.70	5.28	74.4	1.82	25.6	1.68	92.3
60	20	17.9	17.8	7.29	1.69	5.07	69.5	2.22	30.5	1.94	87.4
61	25	17.9	17.8	7.82	1.40	4.52	57.8	3.30	42.2	3.17	96.1
62	10	25.6	25.4	7.25	.55	4.29	59.2	2.96	40.8	2.70	91.2
63	15	25.6	25.4	7.62	1.14	4.53	59.4	3.09	40.6	2.92	94.5
64	20	25.6	25.4	8.00	.92	3.82	47.7	4.18	52.3	3.76	89.9
65	25	25.6	25.4	8.66	.70	3.57	41.2	5.09	58.8	4.49	88.2

In table 3 data are given on the ammoniation at 220°, 200°, and 180° C. In the experiments conducted at 220° the concentrations of ammonia were 12.0, 14.9, and 27.0 percent. The reaction periods ranged from 10 to 25 hours. The data show that the percentage of total nitrogen in the product was only slightly affected by the variation of the concentration of ammonia from 12.0 to 27.0 percent. In each of the three series of experiments the increase in the percentage of total nitrogen was not especially pronounced when the reaction period was increased from 10 to 25 hours. The ammoniacal nitrogen in the product was very little affected by either the change in concentration of ammonia or the variation of the reaction period. The percentages of water-soluble organic nitrogen and of water-insoluble organic nitrogen soluble in neutral permanganate solution were rather irregular in all the three series of experiments. The percentage of water-insoluble organic nitrogen soluble in neutral permanganate solution, expressed as percent of the total water-insoluble organic nitrogen, was generally 80 percent or above.

In the series of experiments conducted at 200° C. the reaction periods also ranged from 10 to 25 hours, and the concentrations of ammonia were 11.4, 14.6, 17.9, and 25.6 percent. As a rule, with the same heating period, the total nitrogen in the product increased with the increase in the concentration of ammonia in the sulphite liquor. However, in experiments 42, 43, 44, and 45, where the concentration of ammonia in the sulphite liquor was 17.9 percent, the percentage of total nitrogen in the product, for some unknown reason, did not show a corresponding increase. Nearly all the experiments recorded in table 3 showed an increase in the percentage of total nitrogen as the period of heating was increased. In practically all the experiments, the ammoniacal nitrogen in the product decreased with the increase in the time of reaction.

In practically all these experiments the percentage of water-soluble organic nitrogen in the ammoniated product as well as the percentage of the water-insoluble nitrogen soluble in neutral permanganate showed a downward trend with the increase in the reaction period from 10 to 25 hours. In the main, the results obtained in experiments 38, 39, 40, and 41, where the concentration of ammonia in the sulphite liquor amounted to 14.6 percent, compare rather favorably as to the percentage of total nitrogen in the ammoniated product and the percentage of water-insoluble organic nitrogen soluble in neutral potassium permanganate solution with those experiments in which sulphite liquor containing higher concentrations of ammonia were employed.

In the experiments conducted at 180° C. the same concentrations of ammonia were used as in the experiments conducted at 200°. The heating periods also ranged from 10 to 25 hours. In practically all these experiments the percentage of total nitrogen in the ammoniated product increased with the increase in the reaction period. However, in the case of the ammoniacal nitrogen in the reaction product, this relationship was reversed, that is, the longer the reaction period the smaller the percentage of ammoniacal nitrogen. This was found to hold also in the experiments recorded in the first two sections of the table. At a temperature of 180° the concentration of ammonia in the sulphite liquor ranged from 11.1 to 25.4 percent without greatly affecting the percentage of total nitrogen in the product. In all

experiments the percentage of water-soluble organic nitrogen decreased steadily with the increase in the reaction period. The percentage of water-insoluble nitrogen soluble in neutral permanganate solution showed a similar tendency, although the results were not so regular and consistent.

AMMONIFICATION AND NITRIFICATION OF NEUTRALIZED AND AMMONIATED RESIDUES OF WASTE SULPHITE LIQUOR

The following experiments [5] were carried out to determine the rate of ammonification and nitrification of the neutralized [6] and ammoniated residues of waste sulphite liquor by soil micro-organisms.

The first series of experiments was conducted for the purpose of ascertaining the relative rates of ammonification of three different preparations of ammoniated waste sulphite liquor [7] as compared with cottonseed meal and casein. For every experiment 25 g of soil in 100 cc of tap water was mixed with a quantity of test substance equivalent to 60 mg of nitrogen, and the mixtures were allowed to incubate for 7 days. The results showed that whereas casein and cottonseed meal were ammonified to the extent of 72 and 18 percent, respectively, the three preparations of ammoniated waste sulphite liquor were ammonified only to the extent of 7, 6, and 5 percent, respectively.

In the second series of experiments the rate of nitrification of a preparation of ammoniated waste sulphite liquor [8] was investigated. The effect of the dry residue of waste sulphite liquor itself, as well as the neutralized residue of waste sulphite liquor, on the nitrification of ammonium sulphate was studied. For each experiment 100-g portions of Keyport soil containing 2 g of calcium carbonate were used, and the mixture was incubated for 1 month under optimum moisture conditions. For the control experiment a quantity of ammonium sulphate equivalent to 30 mg of nitrogen was taken. In other experiments 0.2-, 0.6-, and 1.0-g quantities, respectively, of the dry residue of waste sulphite liquor were employed in addition to the same quantities of ammonium sulphate as was used in the control. The results show that whereas in the control experiment the ammonium sulphate was nitrified to the extent of 84.7 percent, in the experiments where 0.2-, 0.6-, and 1.0-g quantities of the dry residue of waste sulphite liquor had been added, nitrification had proceeded only to the extent of 75.7, 64, and 51.3 percent, respectively, thus indicating that the residue of waste sulphite liquor is definitely toxic to the nitrifying bacteria. When the neutralized residue of waste sulphite liquor was used in place of the unneutralized material, no such toxic effect on the nitrifying bacteria was noted. The ammoniated residue of waste sulphite liquor nitrified in the experimental period of 1 month only to the extent of approximately 20 percent, thus indicating that the nitrogen in this material is rather firmly bound with the organic matter of the waste sulphite liquor. Presumably, upon the destruction of the organic matter the residual nitrogen would become available.

In table 4 data are presented on the relative rates of nitrification of the ammoniated residue of waste sulphite liquor, ammonium sulphate, and blood meal.

[5] All experiments on the ammonification and nitrification by soil micro-organisms recorded in this paper were carried out by N. R. Smith of the Division of Soil Microbiology, Bureau of Plant Industry.
[6] Waste sulphite liquor neutralized with ammonia, evaporated to dryness, and dried at 105° C.
[7] Products from experiments 2, 4, and 10, table 1.
[8] Product from experiment 32, table 3.

TABLE 4.—*Nitrate nitrogen produced by nitrification of ammonium sulphate, blood meal and ammoniated residue of waste sulphite liquor*

Experiment no.	Substance	Nitrate nitrogen found when incubated for—					
		30 days		60 days		90 days	
		Quantity	Percentage of total nitrogen	Quantity	Percentage of total nitrogen	Quantity	Percentage of total nitrogen
		Milligrams	*Percent*	*Milligrams*	*Percent*	*Milligrams*	*Percent*
SANDY LOAM FROM ARLINGTON EXPERIMENT FARM, ROSSLYN, VA.							
1	Ammonium sulphate	24.8	82.7	25.2	84.0	23.4	78.0
2	Blood meal	15.2	50.7	15.3	51.0	12.8	42.7
3	Ammoniated residue of waste sulphite liquor.[1]	6.3	21.0	7.0	23.3	6.0	20.0
4	do.[2]	4.7	15.7	5.6	18.7	5.2	17.3
5	do.[3]	4.5	15.0	4.5	15.0	4.8	16.0
KEYPORT CLAY LOAM FROM WALKER HILL, VA.							
6	Ammonium sulphate	25.0	83.3	25.3	84.3	24.3	81.0
7	Blood meal	12.6	42.0	14.1	47.0	15.2	50.7
8	Ammoniated residue of waste sulphite liquor [1]	5.8	19.3	6.3	21.0	6.0	20.0
9	do.[2]	3.9	13.0	4.6	15.3	5.0	16.7
10	do.[3]	4.7	15.7	4.4	14.7	4.0	13.3

[1] Product from experiment 32, table 3.
[2] Product from experiment 4, table 1.
[3] Product from experiment 6, table 1.

For each experiment 2 g of calcium carbonate and the equivalent of 30 mg of nitrogen were added to 100 g of sifted soil and incubated at 28° C. under optimum moisture conditions. All experiments were carried out in triplicate, and the figures in table 4 in each case represent the mean of three values. In every case a correction was applied for the nitrate nitrogen in the soil.

It will be observed from table 4 that all three preparations of ammoniated waste sulphite liquor nitrified much more slowly than either blood meal or ammonium sulphate. Apparently, in all the materials tested, most of the nitrification occurred in the first 30 days. Blood meal, however, was nitrified from two to three times as rapidly as the three samples of ammoniated waste sulphite liquor.

EVALUATION OF AMMONIATED WASTE SULPHITE LIQUOR AS A PLANT FOOD

A waste material that can be treated so that it will contain approximately 11 percent of nitrogen deserves consideration as a possible plant food material. In view of the fact that the ammoniated waste sulphite liquor is practically free of phosphoric acid (P_2O_5) and the percentage of potash (K_2O) is negligible, the material, so far as being a potential plant food is concerned, is to be valued merely as a source of nitrogen. With a fairly high nitrogen content such a product may possess value for fertilizer or soil-improvement purposes.

While it is true that the nitrogen content of the dry residue of ammoniated waste sulphite liquor is exceeded by a number of inorganic nitrogen materials, such as sodium nitrate and ammonium sulphate, and by certain organic compounds used as fertilizer mate-

rials, including urea and cyanamid, it does compare rather favorably with certain organic nitrogen fertilizer materials, such as castor pomace, with an average nitrogen content of about 5 percent; cottonseed meal, with approximately 7 percent of nitrogen; fish scrap, with 7 to 10 percent of nitrogen; tankage, with a nitrogen content of 5 to 10 percent; and dried blood, with a percentage of nitrogen ranging from 9 to 14.

In order to determine the availability of the nitrogen in the ammoniated residue of waste sulphite liquor to plants, as compared with certain standard nitrogen fertilizer materials, vegetative pot tests were conducted under greenhouse conditions at the Arlington Experiment Farm, Rosslyn, Va. Three nitrogen fertilizer materials were selected for comparative purposes, namely, high-grade dried blood, sodium nitrate, and ammonium sulphate. The tests were conducted in 1-gallon glazed pots capable of holding 5 kg of soil. Norfolk loamy fine sand with a pH of 4.8 was used in the first tests. All tests involving any particular fertilizer treatment were made in triplicate unless otherwise stated. In subsequent tests, a similar soil was used, except that the pH was 5.8 instead of 4.8. Millet was used as the indicator crop in all tests. In the tables, the weights of millet obtained are recorded on a moisture-free basis.

In the first series of pot experiments, ammoniated materials ranging in nitrogen content from 2.60 to 9.07 percent were compared with dried blood and a mixture of sodium nitrate and ammonium sulphate as nitrogen sources. In the sodium nitrate-ammonium sulphate mixture each salt furnished an equal quantity of nitrogen in all the experiments herein reported. The five ammoniated materials were mixed separately with superphosphate (18 percent P_2O_5) and muriate of potash (50 percent K_2O) on a 4–12–4 [9] basis. These five fertilizer mixtures were compared with: (1) A mixture containing only superphosphate and muriate of potash, a 0–12–4 mixture; (2) a 4–12–4 mixture with dried blood as the nitrogen source; and (3) a 4–12–4 mixture with the inorganic salts, sodium nitrate and ammonium sulphate, furnishing the nitrogen. The results of this series of experiments are recorded in table 5.

Table 5 shows that in all vegetative tests a better growth of millet was produced with the ammoniated waste sulphite liquor plus phosphoric acid and potash than with the control, the 0–12–4 mixture. However, in no case was the growth response anywhere near so marked as that resulting from the use of either dried blood or the mixture of inorganic salts as the source of nitrogen in a 4–12–4 mixture.

By giving the 0–12–4 mixture a rating of 100, the mean values of the five series of experiments with the ammoniated residue of waste sulphite liquor, dried blood, and the sodium nitrate-ammonium sulphate mixture are 131, 188, and 196, respectively. The relative order just mentioned reflects the growth response of millet to the different nitrogen sources and clearly indicates a comparatively low availability of the combined nitrogen in the ammoniated materials.

[9] 4 percent of nitrogen (N), 12 percent of phosphoric acid (P_2O_5), and 4 percent of potash (K_2O).

TABLE 5.—*Yields of millet obtained with ammoniated residue of waste sulphite liquor, with dried blood, and with sodium nitrate—ammonium sulphate mixture as sources of nitrogen*

[In all experiments, except in the control, 4–12–4 fertilizer mixtures were employed. Fertilizer was applied at the rate of 2,000 pounds per acre, which is equivalent to 80 pounds of N, 240 pounds of P_2O_5, and 80 pounds of K_2O. Ten millet plants were grown in each pot. Each weight recorded represents 30 plants (moisture-free basis). The soil reaction was pH 4.8.]

Composition of fertilizer N–P_2O_5–K_2O (percent)	Source of nitrogen in fertilizer mixture	Actual and relative yields on an oven-dry basis [1]					
		Series 1		Series 2		Series 3	
		Actual	Relative	Actual	Relative	Actual	Relative
		Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
0–12–4	No nitrogen (control)	21.6	100	17.1	100	19.1	100
4–12–4	Ammoniated waste sulphite material.	[3] 25.7	119	[3] 25.7	150	[4] 28.3	148
4–12–4	Dried blood	43.9	203	30.5	178	39.6	207
4–12–4	Sodium nitrate-ammonium sulphate.	38.5	178	39.0	228	41.4	216

Composition of fertilizer N–P_2O_5–K_2O (percent)	Source of nitrogen in fertilizer mixture	Actual and relative yields on an oven-dry basis [1]					
		Series 4		Series 5		Average	
		Actual	Relative	Actual	Relative	Actual	Relative
		Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
0–12–4	No nitrogen (control)	25.2	100	27.6	100	22.12	100
4–12–4	Ammoniated waste sulphite material.	[5] 29.5	117	[6] 33.1	120	28.46	131
4–12–4	Dried blood	42.1	167	50.9	184	41.40	188
4–12–4	Sodium nitrate-ammonium sulphate.	40.0	159	55. /	200	42.84	196

[1] Series 1 was planted Dec. 29, 1932, and harvested Mar. 30, 1933 (91 days); series 2 was planted Jan. 25, 1933, and harvested Apr. 13, 1933 (78 days); series 3 was planted Feb. 18, 1933, and harvested Apr. 18, 1933 (59 days); series 4 was planted Mar. 20, 1933, and harvested May 6, 1933 (47 days); series 5 was planted Apr. 27, 1933, and harvested June 6, 1933 (40 days).

[2] For this experiment, the residue obtained by neutralizing some waste sulphite liquor with ammonia and then evaporating to dryness was employed; 2.60 percent of nitrogen.

[3] Product from experiment 1, of table 1 used; 7.30 percent of nitrogen.

[4] Product from experiment 2, of table 1 used; 8.32 percent of nitrogen.

[5] Product from experiment 3, of table 1 used; 8.83 percent of nitrogen.

[6] Product from experiment 5, of table 1 used; 9.07 percent of nitrogen.

In view of the fact that the nitrogen in the ammoniated material is rather firmly bound in some organic combination, it was considered desirable to determine whether the material possessed much, if any, residual fertilizer effect. Accordingly, the pots of soil used in series 3 of table 5 were replanted with millet without any fertilizer re-treatment. The roots of the previous millet crop were screened out before replanting. The actual weights of millet obtained, moisture-free basis, were as follows: No nitrogen, 0–12–4 mixture, 12.15 g; ammoniated residue of waste sulphite liquor [10], 4–12–4 mixture, 9.45 g; dried blood mixture, 7.30 g; and inorganic salts mixture, 5.35 g. Expressed on a relative basis with the 0–12–4 mixture taken as 100, the ammoniated material mixture was 78, the dried blood mixture 60, and the inorganic salts mixture 44, thus apparently indicating that the nitrogen in the ammoniated material was available over a longer period than in either the dried blood or the mixture of inorganic salts. However, the comparatively better showing of the 0–12–4 and the ammo-

[10] Product of experiment 2, table 1, which contained 8.32 percent of nitrogen

niated material mixtures was more probably due to the effect of greater residues of phosphoric acid and potash left in the soil. As the soil used in these experiments was particularly responsive to phosphoric acid, and to some extent potash, it would seem reasonable to suppose that the influence of the fertilizer mixtures containing nitrogen, especially when in an available form, would be to promote a heavier growth of millet and thereby remove more available phosphoric acid and potash than in those experiments where the no-nitrogen, 0–12–4, fertilizer mixture was used, or where the nitrogen was not readily available as was the case with the ammoniated material. When replanted without additional fertilizer treatment the presumably greater residues of phosphoric acid and potash in the pots of soil where the 0–12–4 and ammoniated material mixtures were originally applied would help to account for the fact that the greater yields of millet plants were produced where the soil had originally received the more readily available nitrogen materials, namely, dried blood and the mixture of inorganic salts.

In order to eliminate as far as possible the probable effect of unequal residues of phosphoric acid and potash, the following pot experiments were carried out: Four samples of ammoniated residue of waste sulphite liquor were used [11] as nitrogen sources in 4–12–4 fertilizer mixtures. As in the previous experiments, dried blood and a mixture of sodium nitrate and ammonium sulphate were employed as standard nitrogen sources. The same type of soil, Norfolk loamy fine sand, was used as in the previous experiments. The pH of the soil was, however, 5.8 instead of 4.8. In these experiments eight pots of soil were used for each fertilizer treatment and termed "original planting." After the millet was harvested four of the eight pots were replanted without re-treatment; the others were re-treated with a mixture of superphosphate and muriate of potash and then replanted. This was done to overcome as far as possible any deficiency of phosphoric acid and potash that might result through the unequal utilization of these fertilizer constituents by the first millet crop. It was felt that in this way a better idea would be obtained regarding any residual effect of the different nitrogen materials. The results obtained are recorded in table 6.

Table 6 shows that insofar as the original planting experiments (columns 3 and 4) are concerned the results in the main confirm those recorded in table 5. While the several preparations of ammoniated material when mixed with compounds furnishing phosphoric acid (P_2O_5) and potash (K_2O) produced a greater growth of millet than the control, 0–12–4 mixture, the standard nitrogen materials, dried blood and the mixture of sodium nitrate and ammonium sulphate, produced even greater yields.

In the two replanted series it will be noted that replanting without the addition of phosphoric acid and potash reduced the yields of millet considerably. However, the yields from the pots to which the ammoniated material had originally been added as a source of nitrogen in a 4–12–4 mixture were in every case greater than those obtained from the pots which had received either dried blood or the sodium nitrate-ammonium sulphate mixture. However, as already pointed out in connection with the experiments recorded in table 5, the better

[11] Samples 9, 13, and 19 and a preparation made under identical experimental conditions as sample 32 were used. These materials contained 9.92, 8.96, 9.83, and 10.90 percent of nitrogen, respectively.

yields of millet obtained from the control pots, 0–12–4 mixture, and from the pots which had received the ammoniated material as a source of nitrogen were probably due to the effect of greater residues of phosphoric acid and potash.

TABLE 6.—*Yields of millet obtained with ammoniated residue of waste sulphite liquor, with dried blood, and with sodium nitrate-ammonium sulphate mixture as sources of nitrogen in original planting and replanting experiments*

[In all experiments, except in the control, 4–12–4 fertilizer mixtures were employed. Fertilizer was applied at the rate of 2,000 pounds per acre, which is equivalent to 80 pounds of N, 240 pounds of P_2O_5, and 80 pounds of K_2O. Ten millet plants were grown in each pot. All weights recorded represent that of moisture-free material. The soil reaction was pH 5.8.]

Composition of fertilizer N–P_2O_5–K_2O (percent)	Source of nitrogen in fertilizer mixture	Actual and relative yields of millet on an oven-dry basis					
		Original planting (total of 8 pots) [1]		Original planting subdivided and treated [2]			
				Without P_2O_5 and K_2O (4 pots) [3]		With P_2O_5 and K_2O (4 pots) [3]	
		Actual	Relative	Actual	Relative	Actual	Relative
		Grams [4]	*Percent*	*Grams* [5]	*Percent*	*Grams* [5]	*Percent*
0–12–4	No nitrogen (control)	37.15	100.0	4.40	100.0	5.05	100.0
4–12–4	Ammoniated material (9.92 percent N)	53.50	144.0	5.00	113.6	6.35	125.7
4–12–4	Ammoniated material (8.96 percent N)	55.95	150.6	4.00	90.9	6.65	131.7
4–12–4	Ammoniated material (9.83 percent N)	47.6	128.1	4.20	95.4	7.45	147.5
4–12–4	Ammoniated material (10.90 percent N)	58.55	157.6	4.65	105.7	8.60	170.3
4–12–4	Dried blood (13.52 percent N)	71.6	192.7	3.80	86.3	7.30	144.5
4–12–4	Sodium nitrate (high grade)—ammonium sulphate (high grade)	75.2	202.4	2.60	59.1	7.40	146.5

[1] Planted July 26, 1935; harvested Sept. 10, 1935 (46 days).
[2] The "original planting" comprised 8 pots. After the crop of millet was harvested, 4 pots were replanted without adding P_2O_5 and K_2O; the other 4 pots received such treatment, the quantity applied being the same as that applied to the 8 pots in the beginning of the test.
[3] Replanted sets, with and without P_2O_5+K_2O; planted to millet on Sept. 16; harvested Oct. 31, 1935 (45 days).
[4] Average weight of 80 millet plants; 10 plants per pot in all experimental work.
[5] Average weight of 40 millet plants; on the basis of 80 millet plants, the calculated weights would of course be doubled.

In columns 7 and 8, table 6, the results of replanting experiments are presented. In these experiments each pot received phosphoric acid and potash but no nitrogen. It was felt that by this treatment the effect of the residual nitrogen fertilizer material could be better determined. The results show that in only one case was there an appreciably greater residual effect from the ammoniated material, namely, the one which contained 10.90 percent of nitrogen.

Figure 1 shows the millet obtained in a rather typical experiment with ammoniated material, dried blood, and the inorganic salt mixture.

In summing up the results of the greenhouse pot tests, comparing ammoniated waste sulphite liquor, dried blood, and a sodium nitrate-ammonium sulphate mixture as sources of nitrogen for millet when grown on Norfolk loamy fine sand, it may be stated definitely that, while the ammoniated material was effective as a source of nitrogen in causing increased yields of millet, in none of the tests conducted did the increases anywhere near equal those recorded for the standard nitrogen sources.

In considering further studies with the ammoniated waste sulphite liquor it would be of interest to obtain data on its effectiveness as a nitrogen source on different crops, on soils of various texture under field conditions and under diverse climatic conditions; its use as a partial rather than as a sole source of nitrogen; its value for physical purposes in fertilizer mixtures; and its adaptability as a soil mulch or for direct incorporation with the soil.

DISCUSSION

Attempts made in the past to use waste sulphite liquor as a fertilizer material (more correctly as a soil conditioner or as a source of soil organic matter, for the untreated liquor itself has practically no fertilizing value) have proved unsuccessful, largely because the liquor is decidedly toxic to plants. This toxicity has generally been

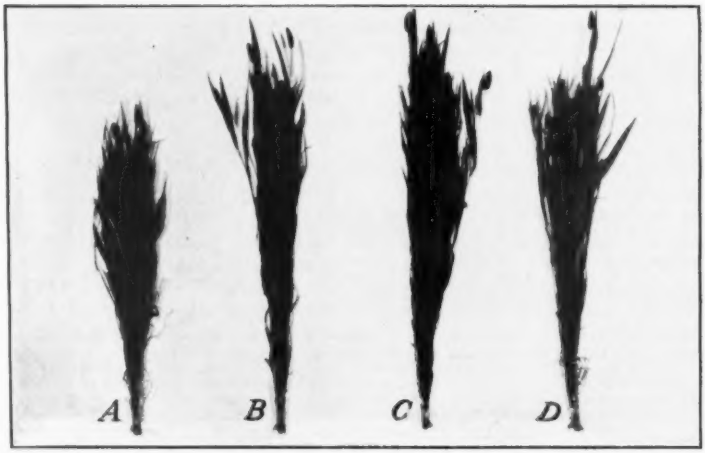

FIGURE 1.—Millet plants (30 in each group) grown in greenhouse pot tests in which different sources of nitrogen were used: *A*, Control (no nitrogen, 0–12–4 mixture); *B*, dried blood; *C*, sodium nitrate-ammonium sulphate; *D*, ammoniated sulphite liquor, 9.92 percent of nitrogen. In *B*, *C*, and *D*, 4–12–4 fertilizer mixtures were used.

attributed to the sulphurous acid and bisulphites in the liquor. The nitrification experiments recorded in this paper show that even the dry residue of waste sulphite liquor (thus presumably free of sulphurous acid) is toxic to the nitrifying bacteria. However, the various products made by heating waste sulphite liquor, or the neutralized residue of this liquor, with aqueous ammonia under pressure were not toxic either to soil micro-organisms or to millet used as the indicator crop in the vegetative tests.

While the percentage of total nitrogen in the ammoniated products became greater with the increase in the temperature of ammoniation, and within certain limits also with the increase in the reaction period, the availability of the nitrogen, as measured by its solubility in water, decreased. The ammoniated materials were ammonified and nitrified rather slowly by soil micro-organisms, as compared with cottonseed meal and casein and with ammonium sulphate and dried blood,

respectively. While the vegetative greenhouse tests indicated that the ammoniated materials caused increased yields of millet, when grown on Norfolk loamy fine sand, they were not so good in this respect as either dried blood or the mixture of sodium nitrate and ammonium sulphate. The replanting experiments also showed that the nitrogen in the ammoniated material is rather firmly combined. Just how the ammonia combines with the lignin sulphonic acids or with the other components of waste sulphite liquor and in what form the nitrogen occurs in the final product can only be surmised. The behavior of the material would suggest that the nitrogen may be present in some cyclic combination.

Attempts were made to apply the method employed in protein analysis for the determination of the distribution of the various forms of nitrogen in the material, but the results obtained could not be satisfactorily interpreted. Furthermore, it was felt that a method developed for the analysis of pure proteins could not be used in the analysis of such a complex material as the ammoniated residue of waste sulphite liquor. The results did show, however, that in some of the ammoniated products only about 30 percent of the nitrogen was converted into soluble form even after boiling with 20-percent hydrochloric acid for 48 hours.

SUMMARY

A study was made of the ammoniation of waste sulphite liquor with aqueous ammonia, under pressure, at temperatures ranging from 180° to 220° C., and under various conditions of concentration of ammonia and time of reaction. The ammoniations were carried out with the neutralized dry residue of waste sulphite liquor (neutralized with ammonia) and with the waste sulphite liquor itself to which various quantities of ammonia had been added. The results show that under a definite concentration of ammonia in the reaction mixture and for any given reaction period, the percentage of total nitrogen in the product increased with the increase of temperature from 180° to 220°. The percentage of total nitrogen in the product was not greatly affected by the variation, within certain limits, of the concentration of ammonia in the reaction mixture.

When 1 part, by weight, of the neutralized dry residue of waste sulphite liquor, was heated at 220° C. for 20 hours with 6 parts, by volume, of 28-percent aqueous ammonia, a product was obtained which contained 10.55 percent of nitrogen. When ammonia was passed into waste sulphite liquor until the concentration of free ammonia was 14.5 percent, and the resulting solution was heated at 220° for 20 hours, the product obtained contained 10.15 percent of nitrogen.

When the ammoniations were conducted at 200° C. or above, the percentage of ammoniacal nitrogen in the products was generally negligible. The percentage of ammoniacal nitrogen in the products obtained from the ammoniation experiments conducted at 180° ranged from 0.55 to 1.92 percent.

While the percentage of water-insoluble nitrogen showed some variation, it generally increased with the increase in the temperature of ammoniation.

The percentage of insoluble nitrogen soluble in neutral permanganate in the ammoniated product was not greatly affected by the temperature of ammoniation.

The ammoniated products obtained were found to be nontoxic to the micro-organisms of the soil, although their ammonification and nitrification were rather slow as compared with that of casein and cottonseed meal and ammonium sulphate and dried blood, respectively.

Vegetative tests conducted in the greenhouse show that as a source of nitrogen the ammoniated material caused increased yields of millet when grown on Norfolk loamy fine sand, but it was not as efficient in this respect as either dried blood or a mixture of sodium nitrate and ammonium sulphate.

Ammoniated waste sulphite liquor may prove to have value as a potential source of nitrogen, as a soil conditioner, or as a source of humus, but as an immediate source of nitrogen for millet it was found to be considerably less effective than either dried blood or a sodium nitrate-ammonium sulphate mixture.

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A STUDY OF THE EFFECT OF BRUCELLA ABORTUS IN UDDERLESS COWS[1]

By B. H. EDGINGTON

Associate animal pathologist, Ohio Agricultural Experiment Station

INTRODUCTION

During the early investigations of *Brucella abortus* as the etiological factor of Bang's disease, the organism was thought to be present only in the uterus, fetus, fetal membranes, and vaginal discharge of the aborting cow. Later, however, Schroeder and Cotton (*20*)[2] reported the finding of abortion organisms in the milk of infected cows, and Fabyan (*9*), Seddon (*23*), Cooledge (*5*), and Giltner and his coworkers (*14*) showed conclusively that the udder is a frequent seat of infection.

While the *Brucella abortus* organism has been isolated from tissues of the cow other than those mentioned (*1*, *8*), the significance of such localizations in relation to perpetuation of the disease remains in question. Gwatkin (*16*) and others have shown the organism to be present in the placentae and vaginal discharges of infected cows at the time of calving, even though abortion did not occur. According to the reports of Cotton (*6*), Schroeder and Cotton (*21*), Schroeder (*19*), and Fitch et al. (*10*), elimination of *Br. abortus* by way of the vagina usually terminates within 60 days after abortion or calving.

Investigations of Giltner and Bandeen (*13*) and Stafseth (*25*) confirm the observation that the nongravid uterus of the *Brucella* aborted cow is not a persistent location of the infection.

The percentage of cows in a Bang's-diseased herd having udder infection varies. Cooledge (*4*) reports udder infection in 27 percent of the animals under observation, Carpenter (*2*) in 66 percent, Gilman (*12*) in 53.7 percent, Schroeder and Cotton (*22*) in 5 percent, Sheather (*24*) in 34 percent, Fitch and Lubbehusen (*11*) in 9 percent, Carpenter and Baker (*3*) in 56.2 percent, and Graham and Thorp (*15*) in 26.1 percent. It is evident from these data that a high rate of udder infection occurs in some diseased herds.

Cotton (*7*) states that the infection may remain in the udder for at least 6½ years, and that this organ seems to be a reservoir from which the uterus can be reinfected. Other investigations support the observation that the udder may retain the infection over long periods of time.

Mitchell and Duthie (*18*) surgically removed the infected udders and supramammary lymph glands from two reactor cows. Following the operations there was a decline in the agglutinin titer of the cows. One showed a recession from 1:600 to 1:25. The other, however, which had an original titer of 1:2,800, still maintained a titer of 1:100 at the termination of the test, 286 days after the udder had been removed. These investigators suggest caution in drawing conclusions from their experiment, although it would appear that re-

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[2] Reference is made by number (italic) to Literature Cited, p. 232.

moval of the infected udders was responsible for the decline in titer which followed the operations.

These references to literature regarding the source and persistence of *Brucella abortus* in affected cows indicate that the udder is the usual seat of localization of the infecting organism in the nonpregnant animal. If the udder is the usual seat of permanent localization of infection in nonpregnant cows, the question arises: Do reactor cows that have aborted and are free of udder infection disseminate *Br. abortus* during subsequent gestations if unbred for 60 to 90 days after aborting and not exposed to other sources of reinfection?

Investigation of this problem requires the use of cows known to be free from udder infection. Diagnosis of udder infection by the use of milk for culture or animal inoculations is not entirely satisfactory as the infected udder may not shed organisms continuously. Similarly, failure to demonstrate *Brucella abortus* agglutinins in the milk is not always a true index of absence of this organism from the udder.

Since available diagnostic methods do not afford unquestionable evidence as to the udder's freedom from *Brucella abortus* infection, an attempt was made in the present experiment to overcome this uncertainty by the use of cows whose udders had been removed surgically.

GENERAL PLAN OF EXPERIMENT

Heifers which showed a negative agglutination reaction to *Brucella abortus*, and whose udders had been removed [3] were raised to sexual maturity in an environment free from *Br. abortus* infection. After reaching maturity they were bred, and when in the fourth to fifth month of gestation they were experimentally exposed to abortion infection. After aborting, these cows were rebred and held under observation until the termination of their second gestation.

Another group of sexually mature heifers, free from Bang's disease, were bred at such intervals that they would be in different periods of gestation at the time the udderless cows were in their fifth to sixth month of pregnancy. Shortly after the cows had been bred for their second gestation, the heifers were added to the group and all were held in the same pasture until 30 days after termination of the last pregnancy.

EXPERIMENTAL ANIMALS

The female cattle used in this experiment were assembled when 4 to 8 months of age, and represented different dairy breeds.

Group U–I (udderless infected) consisted of heifers nos. 24, 30, 31, 32, 34, and 47.

Group U–C (udderless controls) was composed of heifers nos. 33 and 35.

The udders and supramammary lymph glands of these eight heifers were removed surgically when the animals were approximately 6 months of age. These cattle showed a negative agglutination reaction for Bang's disease in monthly tests until they were 22 months old. At that time the heifers of group U–I were experimentally infected with *Brucella abortus*. The heifers of the U–C group were not exposed to the infection.

[3] The operations were performed by Dr. W. F. Guard, College of Veterinary Medicine, Ohio State University, to whom the author wishes to express his appreciation.

Group N–C (normal controls) consisted of six heifers, nos. 2, 3, 4, 5, 6, and 7. The udders of these heifers were intact. The animals were aproximately 8 months old when assembled for this experiment and were about 1 year younger than those of the other groups. These heifers gave negative reactions in agglutination tests for Bang's disease.

Bull A, a grade Holstein of limited service as a sire prior to this experiment, was used in 1932 for breeding the udderless heifers. The animal gave negative reactions to the agglutination test for Bang's disease both before and at the conclusion of his use in this experiment. Bull B, a pure-bred Jersey, was obtained as a yearling from a negative herd. The animal, not used as a sire until about 30 months of age, was employed for all breeding services in this experiment during 1933. This bull gave negative agglutination reactions throughout the experiment.

ASSOCIATION OF GROUPS

The female cattle were pastured during the summer, and stabled with suitable lots for exercise during the winter. They were kept under conditions intended to protect them from exposure to Bang's disease until the time of experimental infection or use as controls for the presence of *Brucella* infection.

The animals of groups U–I and U–C were pastured and stabled together from June 1931 to October 1932. The heifers of these groups were bred during April, May, and June 1932. On October 23, 1932, the animals of group U–I were experimentally infected with cultures of *Brucella abortus*. Immediately prior to this infection the group U–C heifers were moved to the quarters of the group N–C animals.

Following infection each cow of group U–I aborted. These cows were bred again during April and May 1933.

The group U–C cows calved normally and were in direct association with the heifers of group N–C from October 1932 to June 1933, at which time all groups of the experiment were combined. The cows of group U–I had been bred before the groups were united. The cows of the other two groups, with the exception of no. 6 in group N–C (which had been bred in April), were bred at some time during the interval from June to September 1933. After the combination of the three groups the cows were maintained as a unit until the termination of the experiment in August 1934.

EXPERIMENTAL INFECTION

Three strains of *Brucella abortus*, each isolated in 1927 from aborted bovine fetuses, were used for infecting the cows in group U–I. Since their isolation the organisms had been maintained by monthly transfer on nutrient agar media and grew readily under ordinary atmospheric conditions at 37° C. The cultures were identified as bovine strains of *Br. abortus* by the use of dye media as suggested by Huddleson (*17*).

The virulence of the cultures was tested by guinea pig inoculations. The inoculum was a saline suspension of a 48-hour growth of the particular organism on liver agar media and was standardized to the opacity of no. 3 McFarland nephelometer tube. Three guinea pigs

were inoculated with each culture; one received a drop of the inoculum in the right conjunctival sac, another 0.5 cc intraperitoneally, and the other 1.0 cc subcutaneously. The guinea pigs were killed 6 to 8 weeks after inoculation, and on autopsy all but one showed splenic lesions from which *Brucella abortus* was isolated. The agglutinin titers of blood serum from these guinea pigs at the time of death ranged from 1:640 to 1:3,200.

Liver agar slopes were inoculated with the three strains of *Brucella abortus* and incubated 72 hours at 37° C. The growth was removed with sterile saline solution and the suspension adjusted to the opacity of a no. 8 McFarland nephelometer tube. Cows 24, 32, and 34 each received a vaginal inoculation October 23, 1932, of 30 cc of this suspension. The injection was made through a funnel attached to small rubber tubing, the outlet being carried well forward into the vaginal cavity. The inoculum was allowed to gravitate slowly into the vagina, but the entire amount injected was not retained. Cows 30, 31, and 47 each received a conjunctival instillation of two or three drops of the bacterial suspension. Following these inoculations the six cows were allowed to associate in an outdoors enclosure and an adjacent stable. At the time of abortion the cows were put in separate stalls, where they remained until all visible vaginal discharge had ceased. When an abortion occurred in the outside enclosure, the fetus and detached membranes were removed. No other precautions were taken to prevent reinfection of the cows from these sources.

The experimentally infected cows each aborted, within periods of 51 to 95 days after inoculation (table 1). Culture medium was inoculated and guinea pigs were injected with material from the abomasum of each aborted fetus. *Brucella abortus* was recovered from the fetuses of cows 24, 30, 31, and 47.

TABLE 1.—*Breeding and calving records of cows in groups U–I (udderless infected), U–C (udderless control), and N–C (normal control)*

Group and cow no.	Bred	Aborted	Calved	Bred	Calved
U-I:					
24	June 17, 1932	Dec. 13, 1932		May 1 and 22, 1933	Feb. 17, 1934
30	June 8, 1932	Jan. 7, 1932		Apr. 27, 1933	Feb. 10, 1934
31	May 10, 1932	Dec. 30, 1932		May 16, 1933	Feb. 16, 1934
32	June 3, 1932	Jan. 21, 1933		May 6 and Sept. 13, 1933.	June 23, 1934
34	May 20, 1932	Jan. 31, 1933		May 3, 1933	Feb. 7, 1934
47	June 4, 1932	Dec. 10, 1932		May 12, 1933	Feb. 25, 1934
U-C:					
33	Apr. 21, 1932		Feb. 1, 1933	Oct. 12, 1933	July 21, 1934
35	May 4, 1932		Feb. 15, 1933	Aug. 8, 1933	May 18, 1934
N-C:					
2				Aug. 4, 1933	May 15, 1934
3				Sept. 20, 1933	July 8, 1934
4				Aug. 26 and Sept. 17, 1933.	[1]
5				July 1, 1933	Apr. 9, 1934
6				Apr. 30, 1933	Feb. 4, 1934
7				June 9, 1933	Mar. 24, 1934

[1] Did not conceive.

The group U–C, noninfected cows, produced apparently healthy calves at the termination of normal gestation periods.

Before the U–I group of cattle was bred for a second pregnancy, culture medium was inoculated with vaginal material collected from each cow by means of swabs. *Brucella abortus* organisms were not

recovered from any of the inoculations. These cows were bred prior to June 1, 1933, on which date they were turned to pasture in association with the U–C and N–C groups. Cow no. 32 failed to conceive from the service prior to being turned to pasture and was rebred in September. The cows of groups U–C and N–C were bred after the three groups had been combined.

After combination of all groups the cattle were maintained as a single lot until the termination of this experiment in October 1934.

Each of the cows, except no. 4 of group N–C, which failed to conceive, produced an apparently healthy calf at the end of a normal gestation period. Five of the cows in group U–I calved in February 1934, at which time the cows of the other groups were in different periods of gestation, one being in the fourth, two in the sixth, and one each in the seventh, eighth, and ninth month.

AGGLUTINATION TESTS

Results of tests for *Brucella abortus* agglutinins in the blood serum of the various animals, collected at approximately monthly intervals throughout the experiment, are given in table 2. The serums were diluted with antigen so that each tube in the series represented a dilution double that of the preceding. The original dilution was 1:25 and the series of dilutions continued until the agglutinating limit of the serum was reached.

TABLE 2.—*Agglutination records of the cows in groups U–I (udderless infected), U–C (udderless control), and N–C (normal control)* [1]

Group and cow no.	1932										
	Feb. 4	Mar. 12	Apr. 16	May 15	June 23	July 25	Aug. 19	Sept. 19	Oct. 23 [2]	Nov. 20	Dec. 18
U–I:											
24	0	0	0	0	0	0	0	0	0	1,600	800
30	0	0	0	0	0	0	0	0	0	800	800
31	0	0	0	0	0	25−	25−	25	0	1,600	3,200
32	0	0	0	0	0	0	25−	25−	0	800	400
34	0	0	0	0	0	0	0	0	0	400	400
47	0	0	0	0	0	25	25	0	0	400	1,600
N–C:											
7	0	0	0	0	0	0	0	0	0	0	25−

Group and cow no.	1933											
	Jan. 19	Feb. 17	Mar. 18	Apr. 18	May 18	June 18	July 18	Aug. 21	Sept. 20	Oct. 20	Nov. 19	Dec. 21
U–I:												
24	3,200	6,400	3,200−	400−	200−	100−	200−	200−	100−	100−	100	100
30	12,800	6,400	6,400	800	800−	200−	800−	800	400−	800−	800−	400−
31	12,800	6,400	400−	800	1,600−	400−	800−	800	400−	800−	200−	400−
32	3,200	6,400	1,600	800−	400−	100−	400−	400	400−	100−	200−	200−
34	12,800	12,800	12,800	3,200−	6,400	400−	800−	800	400	400	800−	800−
47	3,200	6,400	400−	400−	400	200−	400	400	200−	200	400−	200
U–C:												
33	0	0	25−	0	25−	25−	0	0	0	0	0	0
N–C:												
2	25−	25	25−	25−	0	0	0	0	0	0	0	0
3	25−	0	25−	0	0	0	25−	0	0	0	0	0
4	25−	25−	0	0	0	0	0	0	0	0	0	0
5	25−	0	0	0	0	0	0	0	0	0	0	0

[1] Figures alone represent maximum serum dilutions in which there was complete agglutination. The minus sign (−) following a figure indicates incomplete agglutination in that dilution.

[2] Each cow in group U–I was given a vaginal inoculation of *Br. abortus*.

TABLE 2.—*Agglutination records of the cows in groups U–I (udderless infected) U–C (udderless control), and N–C (normal control)*—Continued

Group and cow no.	1934							
	Jan. 21	Feb. 18	Mar. 18	Apr. 20	May 18	June 19	July 21	Aug. 18
U–I:								
24	200–	100–	100	100	100–	100–	200–	.....
30	1,600–	400–	400	400–	800	200–	200–	.....
31	800–	400	400–	400–	400–	200	400	.....
32	200–	100	200–	200–	200–	200–	200–	.....
34	800	800	800–	400–	400–	200	200	.....
47	400–	200–	200–	200–	200–	100	200	.....
U–C:								
33	0	0	0	0	25–	25–	25–	.....
N–C:								
2	0	0	25–	25–	0	25–	25–	0
3	0	0	0	25–	25–	25–	25–	25–
5	0	25–	0	25–	25–	25–	25–	25–
6	0	0	0	0	25–	0	25–	25–
7	0	0	0	0	0	25–	25–	25–

The antigen used in these tests represented three strains of *Brucella abortus*. Forty-eight-hour growth of these organisms on liver agar slants was removed with phenolized saline solution and the suspension standardized to the opacity of the no. 1 tube of a McFarland nephelometer.

Animals of group U–I did not show agglutinins in their serums, except in a few instances in the 1:25 dilution, until after the animal had been experimentally infected with *Brucella* organisms. Fourteen days after exposure the agglutinin titer of the infected cows ranged from 1:400 to 1:1,600 (not shown in table 2). The peak of these reactions was reached about 60 days after infection, at which time some titers were 1:12,800. During the succeeding 18 months there was a gradual, though somewhat irregular, recession in the limit of the titers. No animal at any time showed a titer lower than incomplete in 1:100; and at the conclusion of the experiment, 21 months after experimental infection, the titers ranged from incomplete in 1:200 to complete in 1:400.

The cows of groups U–C and N–C usually gave completely negative tests. Although agglutination was observed at different times in the 1:25 dilution to some of the blood samples, these reactions were never complete. Failure of these control animals to develop high agglutinin titers was not considered as due to inability of response to antigen stimulation, since four of the cows that later received vaginal inoculations of *Brucella abortus* cultures developed high titers.

Agglutination tests of milk samples obtained from separate quarters of the udders and collected from each cow of the N–C group about 1 week following her calving, were negative. After all the cows of this group had calved, milk was again collected from each for agglutination tests and guinea pig injections. Each sample represented a mixture of milk from all four quarters of the udder. Negative agglutination reactions were obtained with these samples.

Two guinea pigs were inoculated, one intraperitoneally and the other subcutaneously, with gravity cream from milk of each cow. Seven weeks after these injections the guinea pigs were killed for autopsy. Evidence of *Brucella abortus* infection was not obtained in

any of these animals either by post-mortem examination, inoculation of culture medium with spleen tissue, or agglutination tests with blood collected from the guinea pigs at the time of slaughter.

DISCUSSION

A review of the literature shows that some but not all reactor cows having udder infection eliminate *Brucella abortus* in placental membranes at the termination of gestation. Whether reinvasion of the uterus results from the udder infection of the individual cow or comes from other sources, seems not to have been established.

Obviously it is impossible to prove that udder infection would have resulted from the inoculations of *Brucella abortus* given the udderless cows used in this experiment. However, the results obtained by the writer in another test, wherein two of four heifers, aborting after vaginal inoculation with subcultures of the same strains of *Brucella abortus* as were used in the present experiment, showed udder infection, appears to warrant the assumption that at least some of the udderless heifers would have shown udder infection if their udders had not been removed.

The animals used in this test were protected against sources of *Brucella abortus* infection other than the experimental inoculation, but no effort was made to isolate the individual animals as protection against a possible intragroup spread of infection. That the protection was adequate for the conditions of this experiment is apparent from the fact that evidence of *Brucella abortus* infection was not obtained from any of the animals during the period of their association as a single herd.

The significance of agglutinins in the 1:25 dilution of blood serum of the control animals may be open to question, since the minimum titer indicative of infection is a somewhat controversial point. The United States Live Stock Sanitary Association (*26*) recommended for the control of Bang's disease in farm animals, that cattle having agglutinin titers less than complete in the 1:25 dilution be classified as negative, and that those with complete agglutination in the 1:100 or higher dilutions, be considered as positive. Animals giving reactions between these limits are classed as suspicious. The success obtained with measures for abortion control in numerous herds representing thousands of animals, in which agglutination in the 1:25 dilution is not regarded as indicating infection of the animal, seems to justify the opinion that agglutinins in that dilution alone rarely if every indicate an infection of the animal. The failure of the control heifers in any instance to show an increase in agglutinin titers for at least 90 days after the demonstration of agglutinins in the 1:25 dilution tends to support the opinion that active infection was not present in these animals.

Whether the recession but continued maintenance of agglutinin titers within the generally accepted reactor range shown by the experimentally infected cows following abortion, represented a maintained agglutinin production by animals that no loger harbored the infection or whether it was the result of residual infection in some part of the body other than the udder and supramammary lymph glands, remains a question. If the latter is true the cows evidently were not

disseminators of infection during their second gestation period, as judged by nonoccurrence of abortion, nonoccurrence of abortion in the pregnant control heifers directly associated with the udderless reactor cows, failure of agglutinins to develop to a titer generally accepted as indicating infection, negative results of cultural tests with milk and vaginal material collected from these heifers and vaginal material obtained from the udderless cows.

SUMMARY

The object of the experiment herein reported was to determine whether the reactor cow that has aborted but has no udder infection may disseminate *Brucella abortus* during her subsequent gestation.

Cows whose udders and supramammary lymph glands had been removed surgically during calfhood were used in the experiment to eliminate any possibility of the presence of nondetectable *Br. abortus* udder infection in the test animals.

Removal of the udder did not affect gestation, as was demonstrated by the fact that noninfected udderless cows delivered normal calves.

Abortions were induced in udderless heifers by vaginal inoculation with *Br. abortus* (bovine) organisms.

The results give conclusive evidence that the presence of *Br. abortus* agglutinins in the blood of cows is not dependent upon specific udder activity, since each of the infected udderless cows developed a high agglutinin titer. While the height of these titers gradually subsided during the latter part of the experiment, it was always maintained at a point, 1:100 or above, which justified the classification of the animals as positive reactors.

These reactor cows did not eliminate *Brucella* organisms during their second gestation as judged (1) by nonrecovery of organisms from vaginal material of the udderless cows collected within a few hours after their second calving; and (2) by failure of susceptible heifers associated with the udderless cows to abort, to develop specific agglutinins in their blood or milk, or to show *Brucella* organisms in milk or vaginal material collected soon after their calving.

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ROUGH SALMONELLA PULLORUM VARIANTS FROM CHICKS[1]

By H. C. GAUGER

Associate in poultry science, North Carolina Agricultural Experiment Station

INTRODUCTION

Morphological variants among bacteria have been observed by many investigators. The earliest views on variation were crystallized by Nägeli (*4*).[2] Hadley's (*1*) review on "dissociation" leaves no doubt that morphological variability occurs in most bacterial species.

RELATED INVESTIGATIONS

Studies on *Salmonella pullorum* variants were made by Mallmann (*3*), who classified stock cultures into three types; viz, stable smooth, stable rough, and variable rough-smooth or intermediate. A description of these was not given.

Plastridge and Rettger (*6*) have reported on a pleomorphic *Salmonella pullorum*-like organism which was isolated from chicks, adult birds, and eggs laid by the adults that survived a disease outbreak. They found this organism to have fermentative and antigenic characteristics similar to those of *Salmonella pullorum*. They said however, "* * * it differs materially from the well known pullorum disease organism in morphology, cultural requirements, and virulence for adult birds." They (*7*, *p.* [*146*]) also stated that the organism must be considered as a "* * * variant of *S. pullorum* or unusual virulence. This conclusion is further confirmed by the fact that some of the strains isolated from infected birds gradually lost their pleomorphism * * *." The virulence of this organism, however, remained at a comparatively high level even after 6 months' cultivation under artificial conditions. After studying the effect of bacteriophage on 10 stock-culture strains, they reported observations on 3 principal colony types grown on beef-infusion and liver-infusion agar, viz, S_1, ordinary smooth colonies; S_2, colonies that were smooth in appearance but much larger than S_1 colonies; and R colonies, which possessed distinctly roughened surfaces.

The present paper presents data on two rough *Salmonella pullorum* variant strains isolated from chicks.

EXPERIMENTAL SUBJECTS AND PROCEDURE

Three 7-day-old chicks taken from a brood of 240 were sent to the laboratory for autopsy. The house in which the chicks were brooded had not previously been used by poultry for 5 years. This brood of chicks was purchased from a hatchery which obtained eggs from 11 supply flocks. These supply flocks were all officially State blood-tested (stained *Salmonella pullorum* antigen) for pullorum disease

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[2] Reference is made by number (italic) to Literature Cited, p. 238.

carriers before the eggs were collected for hatching purposes. Adult birds in four of these flocks gave a negative reaction in the first test, two flocks gave a negative reaction in the second test, and five flocks were found to contain reactors in the second and final test.

On post mortem the following findings were noted in all three chicks: Yolks—partially absorbed; kidneys—swollen, congested, and containing an abnormal amount of urates; lungs—congested; ureters—distended with urates; proventriculi—thickened mucosa and swollen glands; gizzards—linings eroded and slightly pitted; livers—slightly enlarged, congested, and showing a peripheral gray to yellow pin-point mottling.

Liver tissue from each of the three chicks was streaked to routine nutrient agar (pH 6.8). This medium is known to be satisfactory for the isolation and support of normal smooth stains of *Salmonella pullorum*.

EXPERIMENTAL RESULTS

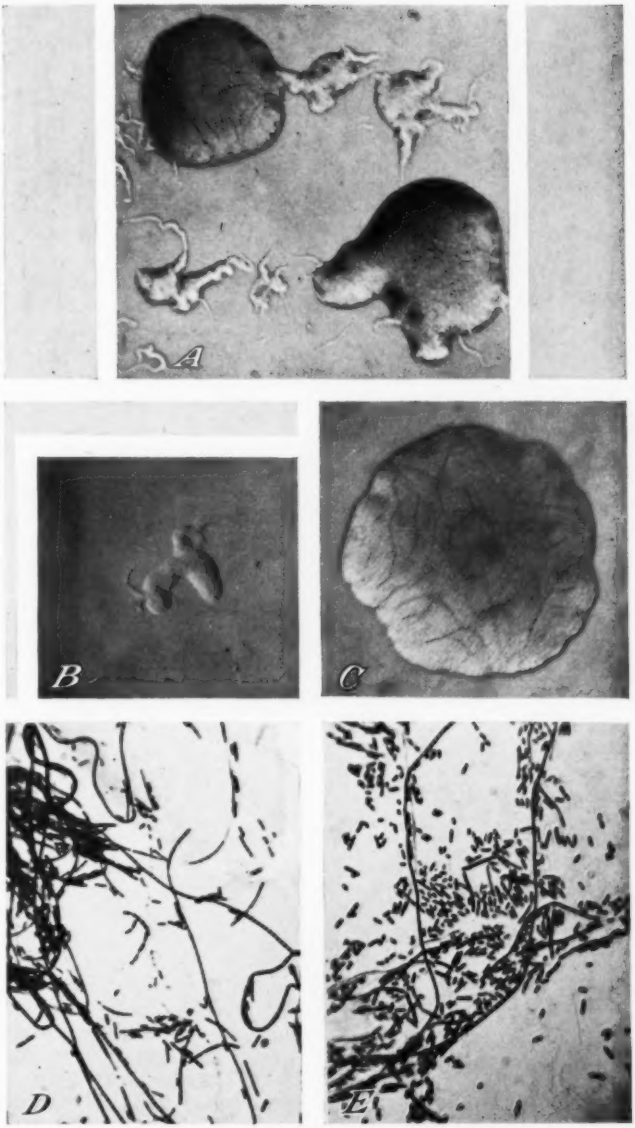
After 24 hours incubation at 37½° C. the agar slants streaked with liver tissue from chicks nos. 1 and 2 showed a few minute colonies, either well-isolated or protruding from a thin, delicate, dull-gray coating of growth which covered a small portion of the agar surface. After failing to show growth at 24, 48, and 72 hours of incubation, the agar slant inoculated with liver tissue from chick no. 3 was discarded.

To facilitate a morphological study, strains 1 and 2, from chicks 1 and 2, respectively, were streaked to a series of Petri dishes containing nutrient agar, and incubated for 24 hours at 37½° C. The plates were relatively free from condensation fluid.

After the inoculated plates had been incubated for 24 hours, strains 1 and 2 appeared indentical. A very thin, delicate, lusterless gray growth was present in areas where the bulk of inoculum had been placed. This growth was seen more clearly when the plates were tilted toward the light. A few small, distinct colonies were also present, some well-isolated and others protruding from the delicate basic growth. These colonies were 0.4 mm or less in diameter and appeared dull gray in reflected light.

A microscopic examination of the cultures (×100 transmitted light) showed the thin, delicate, lusterless gray growth to consist principally of minute, extremely irregularly shaped colonies of various sizes from which projected strands of various lengths and shapes. Frequently single, well-isolated strands constituted a colony. The majority of these colonies were microscopic in size, but a few of the larger ones were barely visible to the naked eye. For convenience in discussing these colonies, the extremely irregular ones are designated the XXR type, and the larger, less irregular, veined colonies, the RRR type. Plate 1, *A* and *B*, shows several typical XXR colonies, and plate 1, *A* and *C*, shows the RRR type. The RRR colonies varied from 0.3 to 0.4 mm in diameter. The borders were more or less wavy and the colony surface distinctly irregular, veined, and granular (transmitted light).

Smears from 24-hour-old XXR colony types, stained with dilute carbolfuchsin, showed extreme chain forms or filaments (pl. 1, *D*). Single rod-shaped cells were present, but chain forms predominated in the microscopic field. Many of these chains extended across several microscopic fields (× 950). Bipolar staining was sometimes



Typical colonies and cells as viewed by transmitted light: *A*, XXR and RRR colonies, × 100; *B*, XXR colony, × 100; *C*, RRR colony, × 100; *D*, cells from XXR colony, × 1,800; *E*, cells from RRR colony, × 1,800.

observed and when present was confined principally to the single cells. However, the uneven staining sometimes seen in the bacterial chains suggests the possibility of bipolar staining or dead bacterial cells within the chains. Single cells varied in size from 0.92μ to 2.6μ in length, and from 0.33μ to 0.54μ in width. The width of the chains varied from 0.33μ to 0.55μ.

Smears from RRR colony types after 24 hours' incubation also showed chain formation (pl. 1, *E*), but they did not show the extremes noted in smears from XXR colonies. Single and double rods in these colonies were more numerous than in smears from XXR colonies. Single rods measured 0.85μ to 2.4μ in length, and 0.3μ to 0.5μ in width. The width of the chains varied from 0.3μ to 0.53μ. Bipolar staining was noted in some of the single and double cells, and an uneven staining in some of the chains.

Organisms from XXR and RRR colony types were gram-negative, nonmotile, and did not produce indole. When tests for H_2S production were made by the method of Jordon and Victorson (*2*) a slight blackening of the culture medium along the line of inoculation was noted after 72 hours' incubation.

Cells from both colony types were inoculated to Dunham's fermentation tubes, such tubes containing 0.75 percent of the test carbohydrate and 1 percent of Andrade's indicator. The inoculated tubes were incubated for 14 days at 37½° C. and produced both acid and gas in tubes containing mannitol, dextrose, arabinose, xylose, rhamnose, levulose, mannose, galactose, and sorbite. Neither acid nor gas was formed in media containing lactose, maltose, sucrose, raffinose, dextrin, inulin, dulcitol, erythritol, salicin, and inositol.

To test the variants under study for autoflocculation, a phenomenon observed by Nicolli (*5*), saline emulsions of a mixture of cells from XXR and RRR colony types were prepared in concentrated and dilute suspensions. These suspensions contained 0.9, 0.5, and 0.1 percent NaCl, respectively, and were preserved with 0.3 percent phenol. After incubation for 40 hours at 37½° C. autoflocculation was not observed, although a slight cell sediment was noted in all tubes, including control tubes, containing the same cell mixture in phenolized distilled water. Upon agitation the sediment arose in a cloudy spiral and became evenly distributed in the test fluid.

To test the agglutinability of the variants an antigen was prepared by suspending a mixture of cells from XXR and RRR colony types in a saline solution containing 0.9 percent NaCl and 0.3 percent phenol. The antigen was adjusted to a turbidity of 1.0 of McFarland's nephelometer scale. After 40 hours' incubation at 37½° C. the antigen was completely agglutinated by a known positive *Salmonella pullorum* serum to a titer of 1 to 400. Partial agglutination occurred in the 1 to 800 and 1 to 1,600 dilutions, but agglutination was not observed in the 1 to 3,200 dilution.

When a similarly prepared antigen was run with negative fowl serum, a distinct clearing, equivalent to a 3+ reading in true agglutination, was noted in dilutions up to and including 1 to 200, 2+ in 1 to 400, and 1+ in 1 to 800 and 1 to 1,600. The partial clearing in the tubes, however, could not be attributed to true agglutination, for on agitation the cell sediment became resuspended without evidence of clumping.

SUMMARY

Studies are presented on two variant strains of *Salmonella pullorum* isolated from baby chicks. These strains varied morphologically and serologically from the normal smooth *S. pullorum* strains usually found in chicks that succumb to the bacteriemic form of *S. pullorum* infection. Both strains exhibited a similar rough colony morphology characterized by two distinct colony types (XXR and RRR types). Antigens prepared from both strains were agglutinated by *S. pullorum* serum and were sensitive to and only partially stable in a saline-negative serum mixture.

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